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THE PREPARATION AND PROPERTIES OF  
NEW CYCLIC HYDROXAMIC ACIDS

by



KENNETH WAYNE HINDMARSH, B.S.P., M.Sc.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
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OF DOCTOR OF PHILOSOPHY

Faculty of Pharmacy and  
Pharmaceutical Sciences

EDMONTON, ALBERTA

SPRING, 1970





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1970  
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UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Preparation and Properties of New Cyclic Hydroxamic Acids" submitted by Kenneth Wayne Hindmarsh in partial fulfilment of the requirements for the degree of Doctor of Philosophy.



## ABSTRACT

Methods to prepare some 3-substituted quinolines have been investigated. Various substituted quinoline N-oxides were prepared by oxidation of the appropriate quinoline. The preparation of cyclic hydroxamic acids by subsequent oxidation of these N-oxides with potassium permanganate in an alkaline medium or with lead tetraacetate is described. Some 3-substituted-1-hydroxy-2(1H)-quinolones, 4-hydroxy-2H-1,4-benzoxazines, -benzothiazines and -benzothiazine 1,1-dioxides have been prepared by reductive cyclization of suitable o-nitro-esters with sodium borohydride catalyzed with palladium-charcoal.

Attempts to convert cyclic hydroxamic acids into thiohydroxamic acids by reaction with phosphorous pentasulfide were unsuccessful.

The mass spectra of some 3- and 4-substituted quinoline hydroxamic acids and related compounds and various benzoxazine and benzothiazine hydroxamic acids and related lactams have been recorded and interpreted. Most of the quinoline hydroxamic acids showed strong  $(M-16)^+$  ions and weak  $(M-17)^+$  ions. The expulsion of CO and HCN molecules and H and HCO radicals were common subsequent decompositions. The spectrum of 4-hydroxy-2-methylquinazoline-3-oxide was unique and showed that nitric oxide was expelled from the molecular ion. The benzoxazine hydroxamic acids fragmented initially by expelling an oxygen atom and a COOH rad-





## ACKNOWLEDGEMENTS

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TO LOIS



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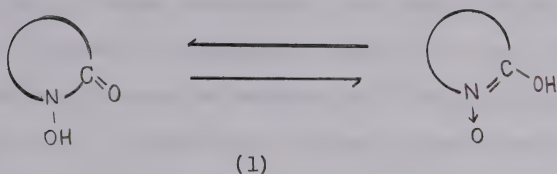


## INTRODUCTION



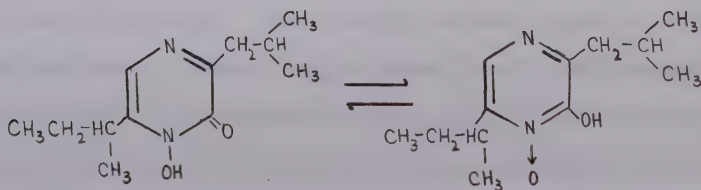
## INTRODUCTION

It is convenient to think of cyclic hydroxamic acids as being N-hydroxy lactams (1), (Coutts, 1967a). Compounds



possessing the hydroxamic group are capable of tautomerism; if cyclic, they are relatively strong organic acids, soluble in sodium bicarbonate solution, and when an alcoholic solution of ferric chloride is added to a solution of the hydroxamic acid in alcohol, a characteristic magenta color is formed. This qualitative test is often employed to confirm the presence of a hydroxamic acid.

The discovery of the antibacterial properties of aspergillic acid (2) by White in 1940, precipitated a





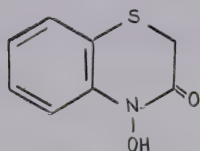
chemical search for proof of the structure of aspergillic acid and for methods of synthesizing related compounds. The knowledge that aspergillic acid was a derivative of pyrazine N-oxide, substituted in the 2-position by a hydroxy group resulted in the development of a variety of procedures for the preparation of acyclic and cyclic hydroxamic acids. These procedures have been the subject of two recent reviews by Coutts in 1967 and so only preparations involving oxidative methods will be considered now in any detail. In addition, the mass spectrometry of aromatic N-oxides and hydroxamic acids and the antibacterial activity of hydroxamic acids will be discussed.

The most important methods of preparing cyclic hydroxamic acids other than by oxidative methods are a) reductive cyclizations, b) synthesis by means of ring expansion, and c) condensation reactions.

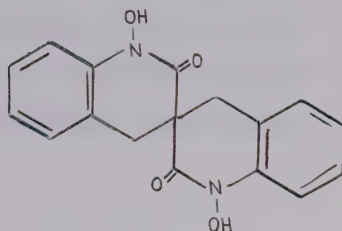
Coutts and Wibberley in 1963 were able to show that o-nitro esters, in which the ester group is suitably orientated with respect to the o-nitrophenyl group, are converted in good yields to cyclic hydroxamic acids on treatment with sodium borohydride in the presence of palladium-charcoal. This method seemed capable of wide application. Various ring systems have been prepared by this method. For example, Coutts et al (1964) found that reductive cyclization of methyl  $\alpha$ -(o-nitrophenylthio)acetate with sodium borohydride and palladium-charcoal gave 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine (3). A



similar reduction of ethyl di(o-nitrobenzyl)malonate yielded the first spiro-cyclic hydroxamic acid, 3,3'-spirobi-(1,2,3,4-tetrahydro-1-hydroxy-2-oxoquinoline) (4).



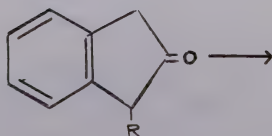
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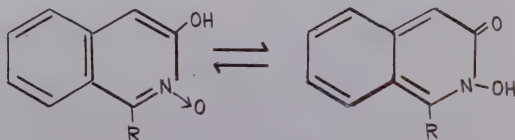
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Similar reductive cyclizations can be effected by employing zinc and ammonium chloride as the reducing system (Honkanen and Virtanen, 1960).

There are relatively few examples of the preparation of cyclic hydroxamic acids by means of ring expansion. Blomquist and Moriconi (1961) found that nitrosation of 1-alkyl-2-indanones (5) under alkaline conditions resulted in ring expansion and produced 1-alkyl-3-hydroxy-isoquinoline-2-oxides (6).



(5)

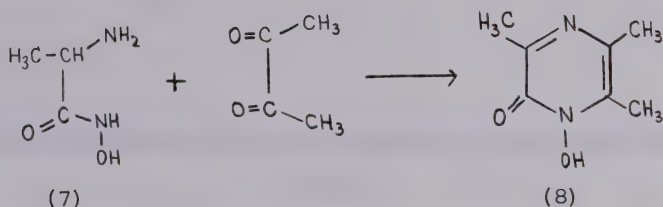


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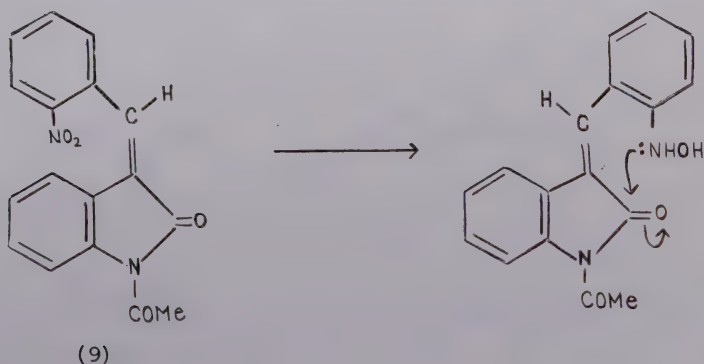




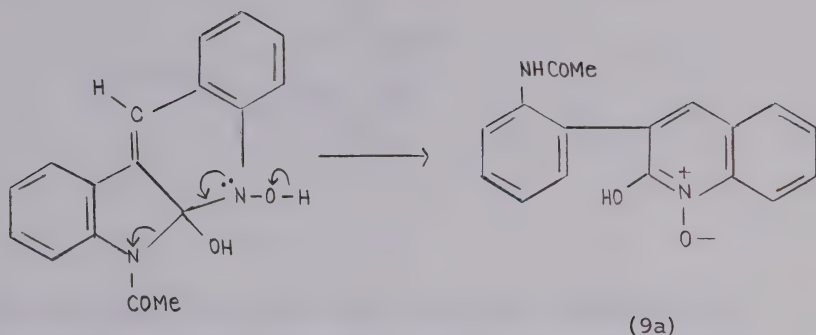
Condensation of  $\alpha$ -aminohydroxamic acids with 1,2-dicarbonyl compounds in the presence of sodium hydroxide yields cyclic hydroxamic acids (Safir and Williams, 1952; Dunn et al, 1949). Treatment of alanine hydroxamic acid (7) with diacetyl, for example, gave the cyclic hydroxamic acid 1,2-dihydro-1-hydroxy-3,5,6-trimethyl-2-oxopyrazine (8).



Abramovitch et al (1967) have described the formation of a hydroxamic acid (9a) as a result of the sodium borohydride-palladium-charcoal reduction of cis-N-acetyl-o-nitrobenzylideneoxindole (9).





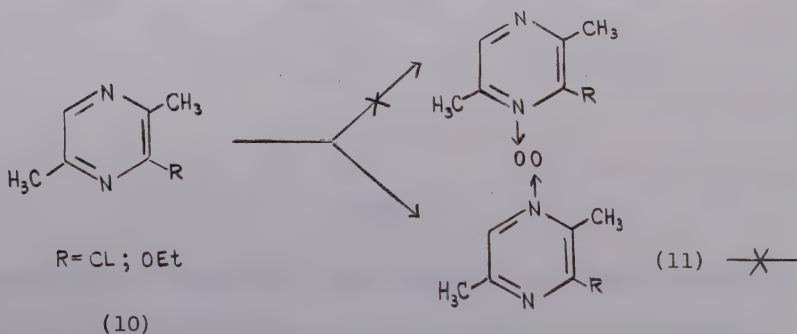


## Preparation of Cyclic Hydroxamic Acids by Oxidative

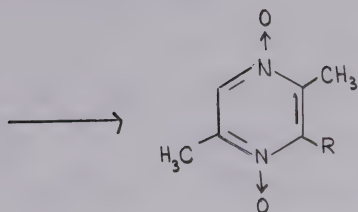
### Methods

#### Oxidation by means of Hydrogen Peroxide and Peracids

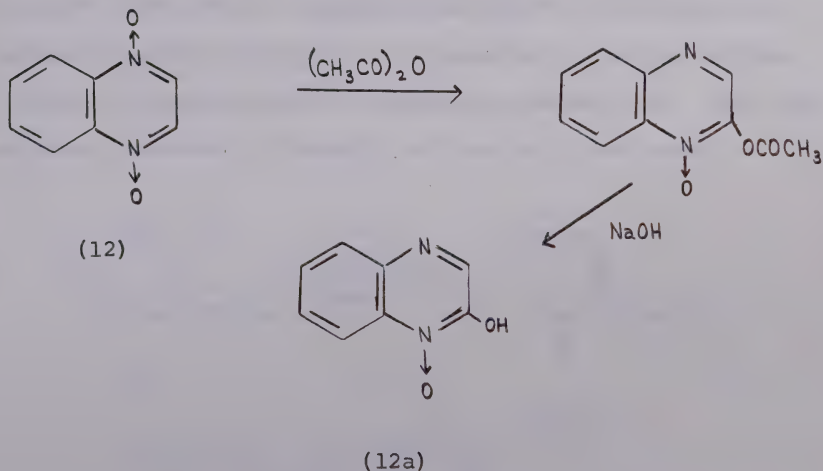
In 1948, Baxter, Newbold and Spring investigated the oxidation of several pyrazine derivatives in an attempt to prepare cyclic hydroxamic acids related to aspergillic acid. The oxidation of 2-chloro- and 2-ethoxy-3,6-dimethylpyrazine (10) with hydrogen peroxide resulted in the 4-monoxide only (11). The product resisted further







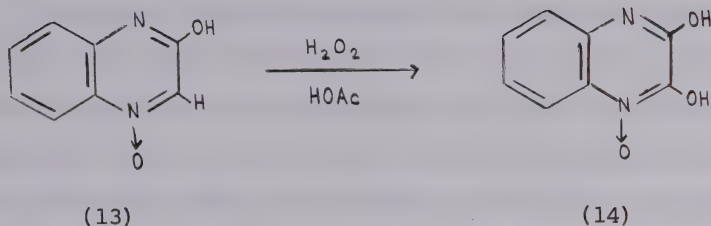
oxidation at the 1-position and so it was concluded that synthesis of a cyclic hydroxamic acid from a 2-substituted pyrazine derivative by direct oxidation was impractical. Elina (1962), however, has shown that treatment of quinoxaline 1,4-dioxide (12) with acetic anhydride and then sodium hydroxide, yielded the hydroxamic acid, 2-hydroxyquinoxaline 1-oxide (12a). The oxidation of 3-hydroxy-



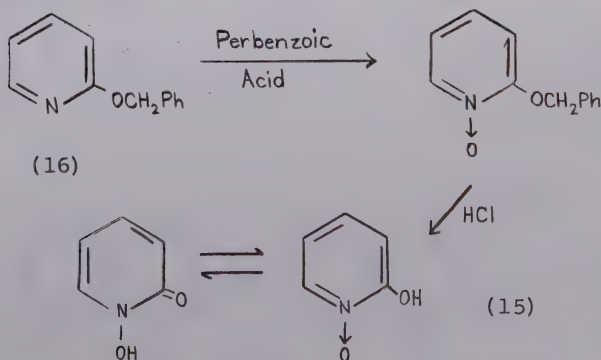
quinoxaline 1-oxide (13) with hydrogen peroxide and acetic acid was shown by Tennant in 1963 to give 2,3-dihydroxy-



quinoxaline 1-oxide (14).



Shaw (1949), in his attempts to develop synthetic methods for introducing the hydroxamic acid grouping into heterocyclic rings, synthesized 1-hydroxy-2(1H)-pyridone (15) (1,2-dihydro-1-hydroxy-2-oxopyridine). The synthesis was achieved by the conversion of 2-benzoyloxy-pyridine (16) to its N-oxide, by means of perbenzoic acid. This oxide was then dissolved in aqueous hydrochloric acid which resulted in rapid hydrolysis as indicated by the separation of benzyl chloride and the crystalline acid (15). The same acid was obtained by Newbold and Spring (1948) when 2-

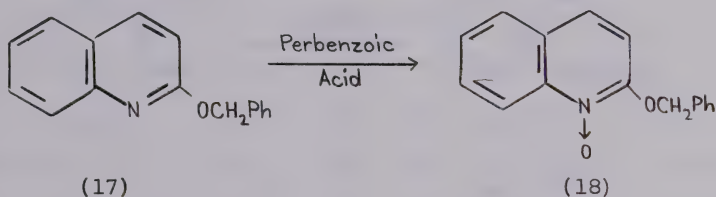




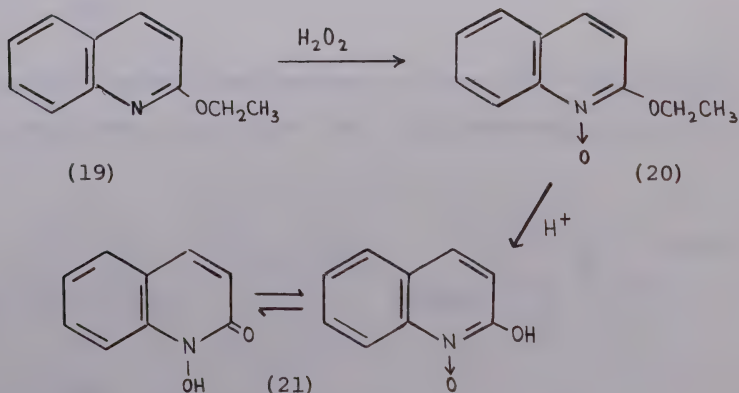


ethoxyquinoline was treated with hydrogen peroxide and acetic acid, followed by acid hydrolysis.

Because 1-hydroxy-2(1H)-pyridone showed antibacterial activity, Lott and Shaw in 1949 extended their synthetic investigations to the preparation of additional hydroxamic acids. Treatment of 2-benzyloxyquinoline (17) with perbenzoic acid did not give satisfactory yields of 2-benzyloxyquinoline-1-oxide (18). However, Newbold and Spring (1948) reported that 2-ethoxyquinoline (19) when

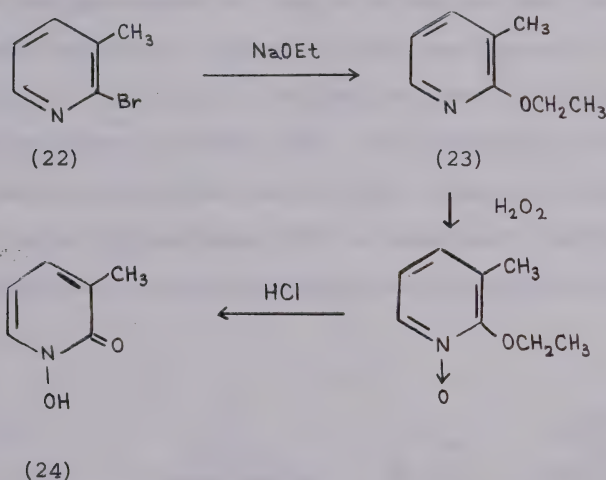


treated with hydrogen peroxide yielded 2-ethoxyquinoline 1-oxide hydrate (20) and hydrolysis of this oxide with mineral acid gave 1-hydroxy-2(1H)-quinolone (21).

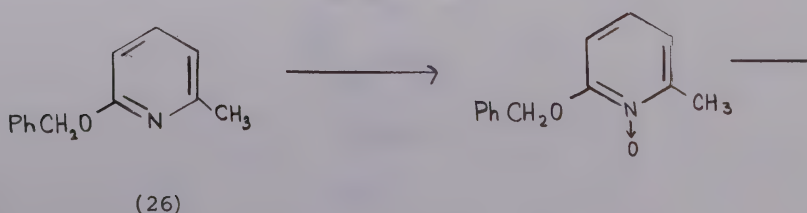




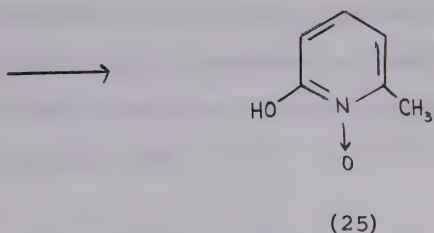
Further syntheses of pyridine and quinoline derivatives of hydroxamic acids have been described by Cunningham et al (1949). 2-Bromo-3-methylpyridine (22) was converted into 2-ethoxy-3-methylpyridine (23) by treatment with sodium ethoxide. This product was then oxidized with hydrogen peroxide and the product was hydrolyzed with dilute mineral acid to give 1-hydroxy-3-methyl-2(1H)-pyridone (24). Similarly, Adams and Miyano (1954) were able to



obtain the 6-methyl derivative (25) by first treating 6-benzyloxy-2-methylpyridine (26) with peracetic acid and

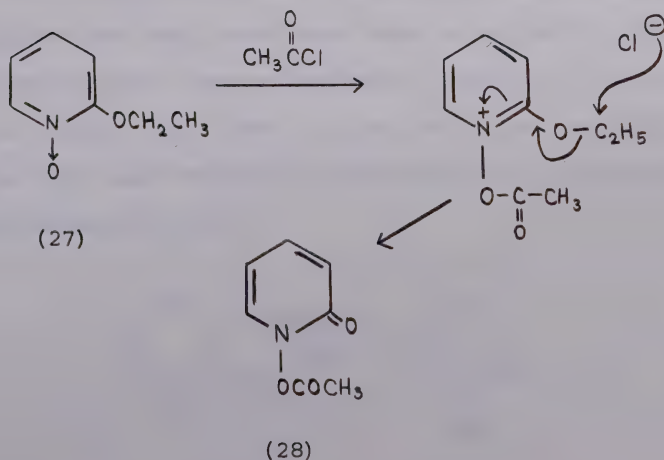






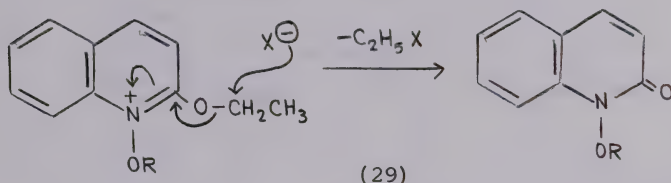
then debenzylating either by hydrolysis with hydrochloric acid, or by hydrogenation over a palladium catalyst.

More recently, Paquette (1965) has reported the result of an electrophilic attack of acetyl chloride on 2-ethoxypyridine 1-oxide (27). This reaction proceeds exothermally with evolution of ethyl chloride to produce 1-acetyloxy-2(1H)-pyridone (28) in excellent yields. Dissolution of the acetyl derivative in a minimum quantity

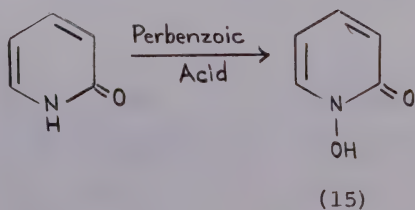




of water at room temperature resulted in the quantitative deposition of colorless crystals of 1-hydroxy-2(1H)-pyridone (15). Paquette in 1966 extended this reaction to the preparation of substituted pyridones and related quinolones (29).



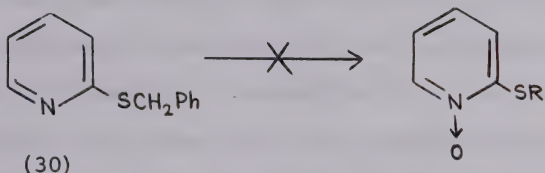
Direct oxidation of amides to hydroxamic acids by means of hydrogen peroxide and ferric chloride was reported by Oliveri-Mandala (1922). Conversion of 2(1H)-pyridone to the related hydroxamic acid, 1-hydroxy-2(1H)-pyridone (15), is a similar reaction. This oxidation was achieved by Lott and Shaw in 1949. Persulfuric acid, perbenzoic acid and performic acid have been tried but of these only perbenzoic acid gave satisfactory results. In the quinoline series, less than 1% yield of 1-hydroxy-2(1H)-quinolone (21) was obtained on treatment of 2(1H)-quinolone with perbenzoic acid.



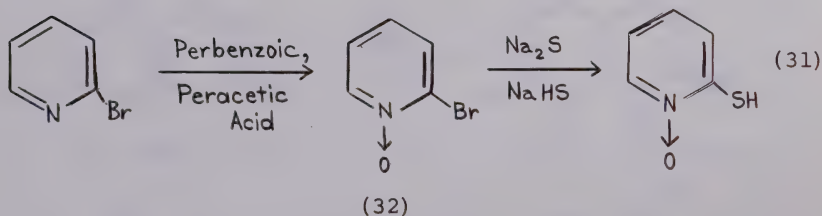




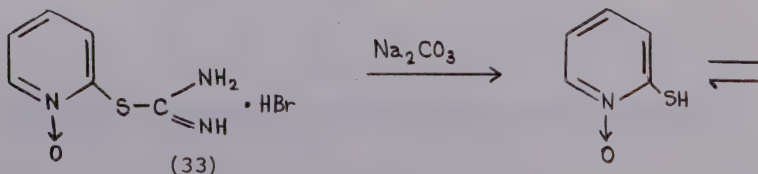
Shaw et al (1950) were unsuccessful in their attempts to synthesize the sulfur analog of 1-hydroxy-2(1H)-pyridone (15) by oxidation of 2-benzylmercaptopyridine (30). Their lack of success was due to the ease of oxidation of the sulfur atom to the electron-withdrawing sulfoxide group.



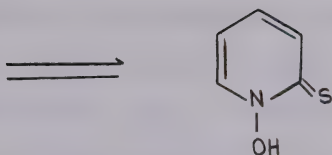
Oxidation at the nitrogen atom had to be accomplished before introduction of the sulfur moiety into the molecule (31). The reaction was also accomplished when 2-bromo-



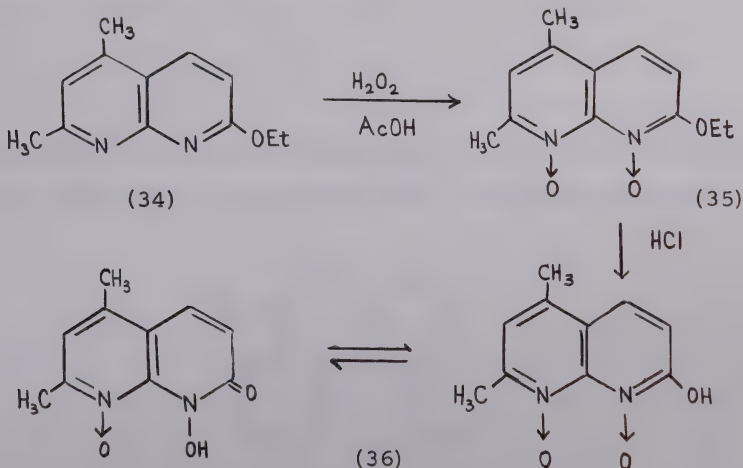
pyridine N-oxide (32) was reacted with thiourea to give initially 2-pyridyl-N-oxide isothiurea hydrobromide (33) which was then treated with aqueous sodium carbonate.







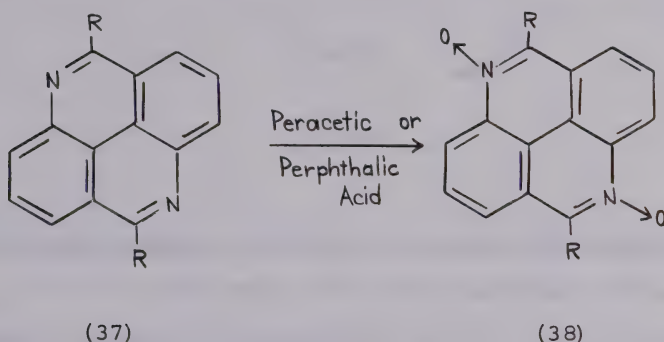
Colonna and Runti (1952) reported a synthesis of cyclic hydroxamic acids derived from 1,8-naphthyridine. 2-Ethoxy-5,7-dimethyl-1,8-naphthyridine (34) was heated under reflux for three hours with hydrogen peroxide and glacial acetic acid and this gave 2-ethoxy-5,7-dimethyl-1,8-naphthyridine 1,8-dioxide (35), which upon hydrolysis with hydrochloric acid was converted to 2-hydroxy-5,7-dimethyl-1,8-naphthyridine 1,8-dioxide (36).



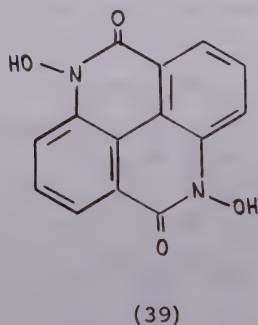
Oxidation by means of peracids has been applied to



larger ring systems. Robbins (1960) reported the smooth oxidation of 5,10-dimethyl- and 5,10-diphenyl-4,9-diazapyrene (37) to the di-N-oxides (38) by means of peracetic or perphthalic acids. Attempts to prepare each mono-N-oxide gave only dioxide and unchanged diazapyrene. Oxidation of 4,9-diazapyrene by peracetic acid gave no oxide



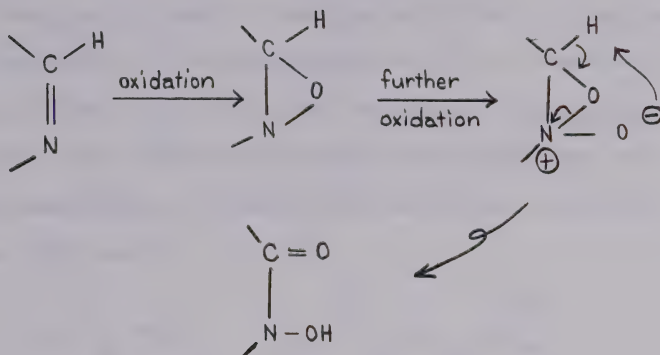
but instead a high melting product to which the dihydroxamic structure was assigned (39). Gawlak and Robbins



have postulated the following mechanism for the formation

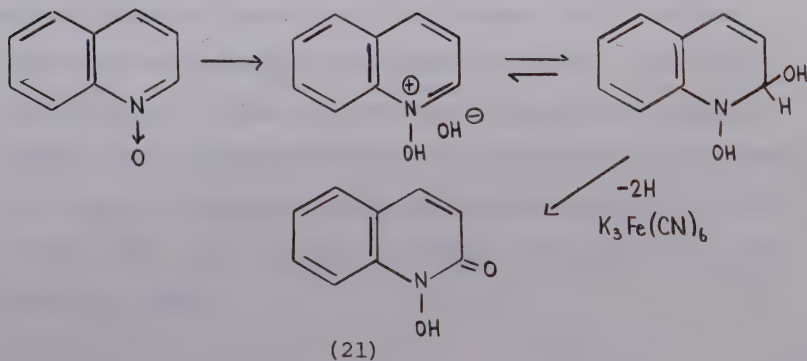


of the hydroxamate.



#### Oxidation by Means of Alkaline Potassium Ferricyanide

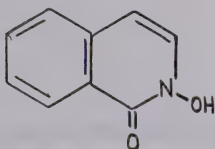
It is a well known fact that quaternary salts of aromatic nitrogen heterocycles, such as N-substituted pyridinium and quinolinium salts, are oxidized by alkaline ferricyanide to N-substituted  $\alpha$ -oxo compounds (Hamana and Yamazaki, 1962). In 1962, Hamana and Yamazaki reported the formation of 1-hydroxy-2(1H)-quinolone (21) in 75% yield by the oxidation of quinol-



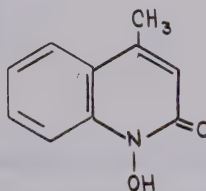




ine 1-oxide with potassium ferricyanide and potassium hydroxide. This yield can be contrasted with the low yield obtained by Newbold and Spring (1948) by the peroxide oxidation of 2-ethoxyquinoline (19). Hamana and Yamazaki (1962) also obtained 2-hydroxy-1(2H)-isoquinolone (40) and 1-hydroxy-4-methyl-2(1H)-quinolone (41) by the same method, although the latter was isolated only in 20% yield.



(40)

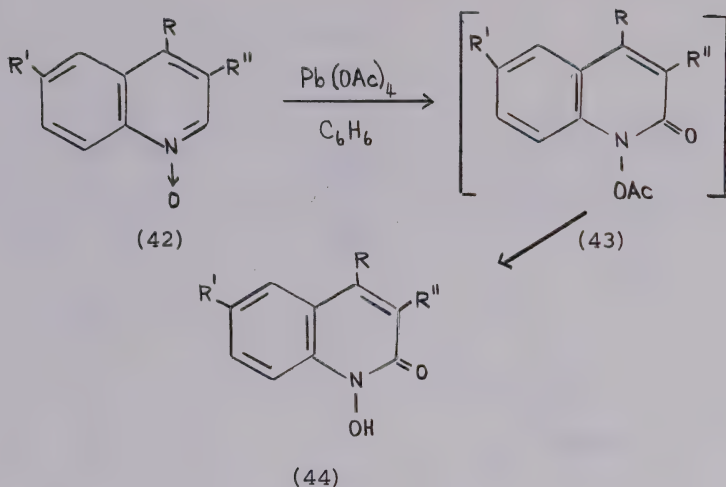


(41)

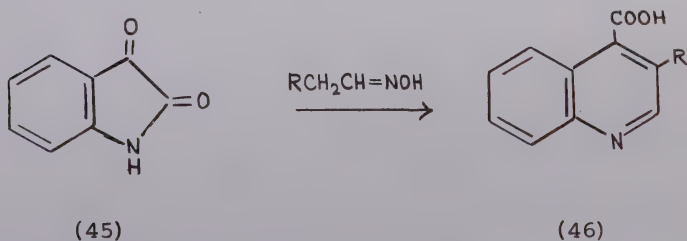
#### Oxidation by Means of Lead Tetraacetate

Ohta and Ochiai (1962) found that 4-methylquinoline 1-oxide (42;  $R=CH_3$ ,  $R'=R''=H$ ) was converted to 1-acetoxy-4-methyl-2(1H)-quinolone (43;  $R=CH_3$ ,  $R'=R''=H$ ) when the former was treated with lead tetraacetate in benzene. This product on hydrolysis with mineral acid yielded 1-hydroxy-4-methyl-2(1H)-quinolone (44;  $R=CH_3$ ,  $R'=R''=H$ ), in 81% yield. Other derivatives prepared in a similar manner were 1-hydroxy-6-methyl-2(1H)-quinolone (44;  $R=R''=H$ ,  $R'=CH_3$ ), 4-chloro-1-hydroxy-2(1H)-quinolone (44;  $R'=R''=H$ ,  $R=Cl$ ), and 3-bromo-1-hydroxy-2(1H)-quinolone (44;  $R=R'=H$ ,  $R''=Br$ ).

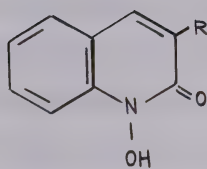
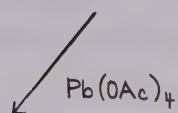
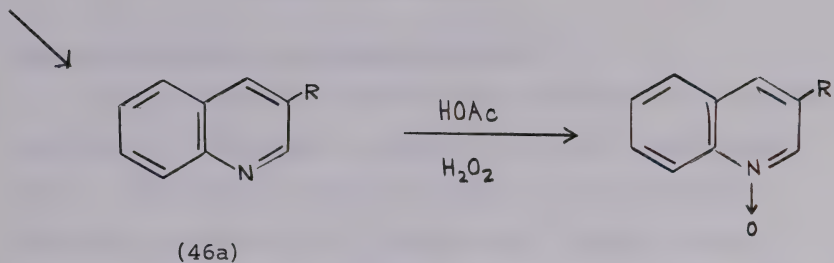




Coutts, Pitkethly and Wibberley (1965) have shown that the interaction of isatin (45) and a suitable aldoxime gave a 3-alkylquinoline-4-carboxylic acid (46), which could be decarboxylated to a 3-alkylquinoline (46a). The product, when successively oxidized with hydrogen peroxide, then lead tetraacetate was converted to the corresponding 3-alkyl-1-hydroxy-2(1H)-quinolone (47).







(47)



## Mass Spectrometry

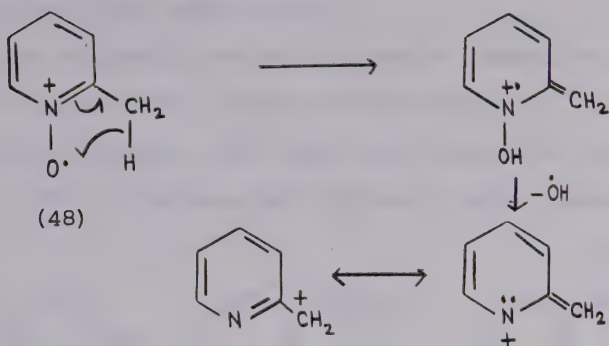
### Mass Spectrometry of Aromatic N-oxides

It is difficult to detect the presence of an N-oxide function in a molecule by means of infrared spectrophotometry. The N-oxide stretching bands of the aromatic N-oxides are located in the 'fingerprint' region between 1300 and 1200  $\text{cm}^{-1}$ . According to Bryce and Maxwell (1965), mass spectrometry easily solves this problem, even if a nitro group is present.

The first study of the fragmentation of aromatic N-oxides upon electron impact was done by Bryce and Maxwell in 1965. These authors studied a few quinoline N-oxides to determine whether or not abundant  $(M-16)^+$  or  $(M-17)^+$  ions were present. They found that quinoline N-oxides give  $(M-16)^+$  ions of 15-40% abundance. This work prompted Grigg and Odell (1966) to report the results obtained by them when  $\Delta'$ -pyrroline N-oxides and pyridine N-oxides were subjected to electron impact. The pyridine N-oxides all showed abundant  $(M-16)^+$  ions but the abundance of this ion is drastically decreased by alkyl substitution in the 2-position due to the operation of an ortho-effect. Thus, the base peak in the spectra of 2-alkylpyridine N-oxides was the  $(M-OH)^+$  ion. An example of the ortho-effect is depicted for 2-methylpyridine N-oxide (48).







$\Delta'$ -Pyrroline N-oxides (49, in which the substituents are H atoms or methyl groups), in sharp contrast to aromatic N-oxides, show much lower abundances of  $(M-16)^+$  ions (1-10%) and hence the  $(M-16)^+$  ion is not of diagnostic value for these compounds.

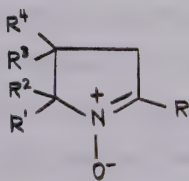
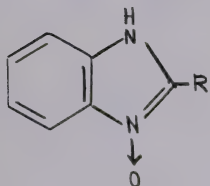


Bild and Hesse in 1967 reported a further study on the mass spectrometry of pyridine N-oxides, in which they dealt with various 2-, 3- and 4-substituted derivatives. Their main concern was again the abundances of the  $(M-16)^+$  and  $(M-17)^+$  ions, which varied considerably depending on

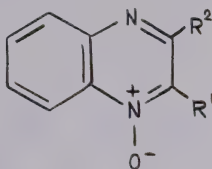


the nature of the substituents.

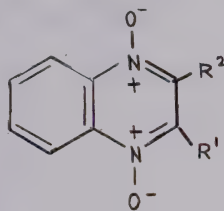
The diagnostic value of electron impact on benzimidazole N-oxides (50), quinoxaline N-oxides (51), and quinoxaline N,N-dioxides (52) has been reported by Tatemutsu et al (1967). The base peak of most 2-alkyl-substituted



(50)



(51)



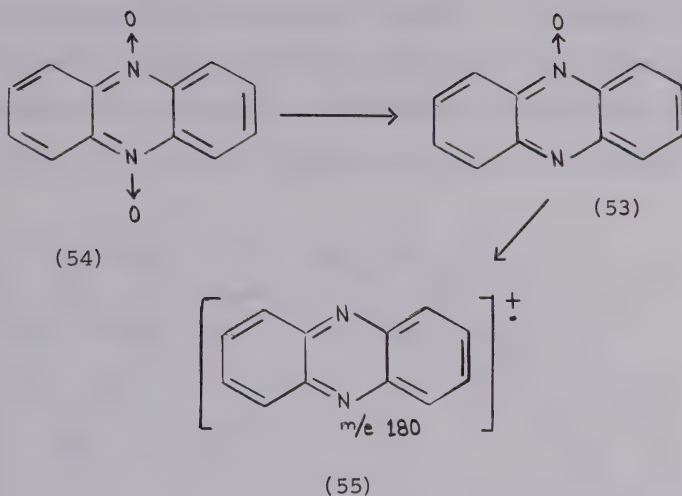
(52)

benzimidazole N-oxides as well as benzimidazole N-oxide is the  $(M-16)^+$  peak. This results from the elimination of an oxide oxygen atom from the molecular ion. In sharp contrast to the benzimidazole N-oxides, the base peak in the spectra of quinoxaline N-oxides (51) is due to an  $(M-17)^+$  ion. The ortho-effect seems to be quite prominent with the latter. Quinoxaline N,N-dioxides (52), however, show preferential loss of an oxygen atom from the molecular ion rather than expulsion of an OH radical through the ortho-effect of the alkyl groups.

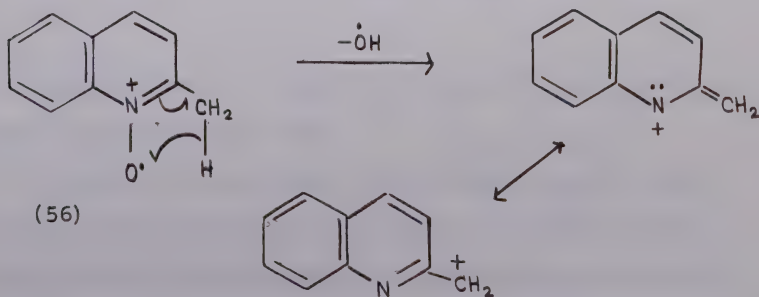
Morita (1966) found that there was no marked difference among the spectra of the phenazines. In the spectra of the mono-N-oxide (53) and the di-N-oxides (54), deoxygenation takes place in a stepwise mode to give the phenaz-



inium cation,  $m/e$  180, (55) which is the base peak in the spectrum.

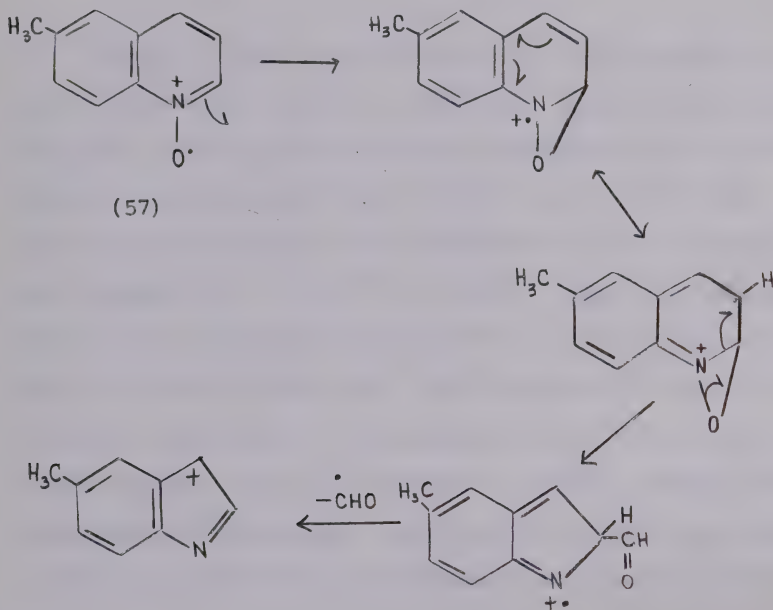


The result of an ortho-effect is observed in the mass spectra of 2-substituted quinoline N-oxides and iso-quinoline N-oxides. Butchardt et al (1968) found that 2-methylquinoline N-oxide (56), like 2-methylpyridine N-oxide (48), expelled an OH radical from the molecular ion. This  $(M-17)^+$  ion is a major fragment.





In contrast, the major fragment derived from 4-methyl- and 6-methylquinoline N-oxide (57) is the result of the loss of 29 mass units ( $M-CHO$ )<sup>+</sup>. It appears that the distant methyl substituent must be influencing the fragmentation process. It has been proposed that the loss of a CHO radical proceeds as illustrated below.

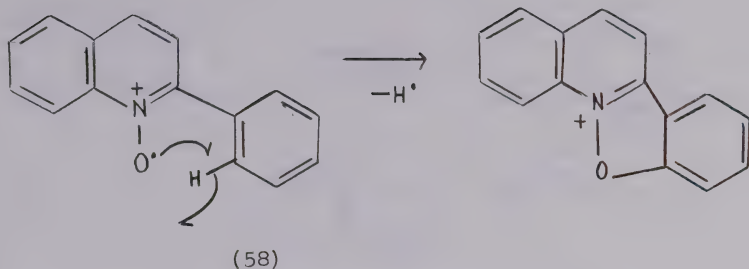


An ( $M-1$ )<sup>+</sup> ion is formed on electron impact of 2-phenylquinoline N-oxide (58).

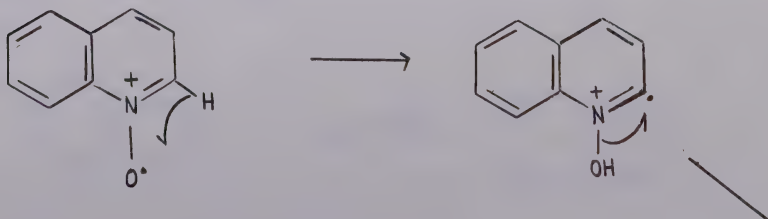
It is easy to conclude, as Butchardt et al (1968) did, that mass spectra can be of great diagnostic value in the structure determination of heteroatomic N-oxides.



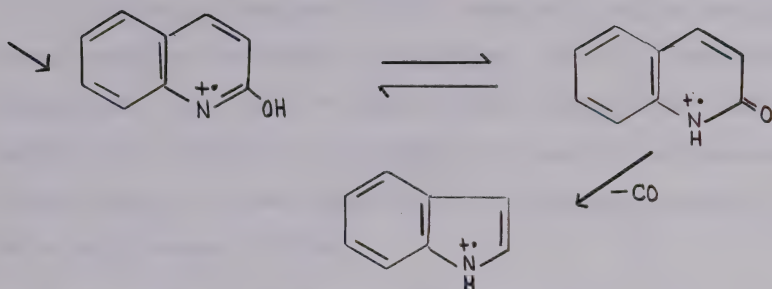




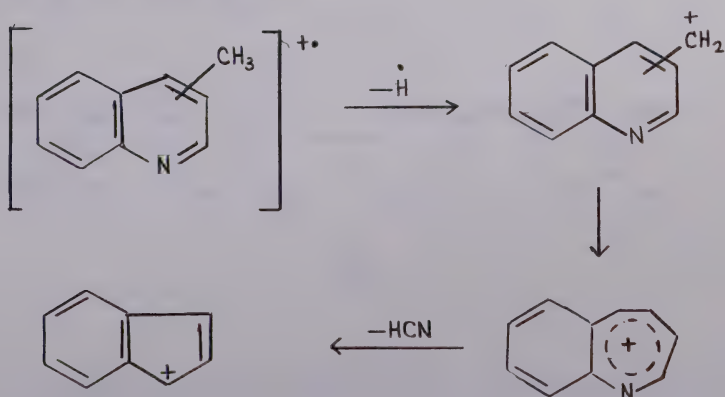
Coutts (1968) has confirmed that rearrangements do occur when the N-oxides of quinoline, isoquinoline and lepidine (4-methylquinoline) are bombarded with electrons. These compounds showed both  $(\text{M}-16)^+$  and  $(\text{M}-17)^+$  ions. In each case the presence of a metastable ion of appropriate mass supported an  $(\text{M}-16)^+ \longrightarrow (\text{M}-17)^+$  loss of a hydrogen atom, thus confirming that the  $\text{M}^+ \longrightarrow (\text{M}-17)^+$  transition was a two-step elimination. The formation of many of the ions was the result of the expulsion of HCN molecules from other fragment ions. In addition, however, unexpected fragmentations occurred, and they can be explained only in terms of molecular rearrangements such as the following:







The initial loss of an HCN molecule has also been observed in the mass spectra of quinoline and isoquinoline (Sample et al, 1967). Sample et al (1967), observed that the major fragmentation of monomethylquinolines proceeds through the sequence  $M \longrightarrow (M-H) \longrightarrow M-(H+HCN)$ . Since they found that the  $M-(H+HCN)/(M-H)$  ratio was constant for the 2-, 3-, and 4-isomers, they concluded that the loss of HCN must proceed from a common intermediate, which they postulated to be the azatropylium ion (59).



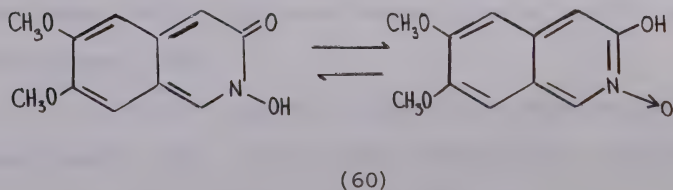
(59)



Draper and MacLean (1968) obtained similar results when studying the effect of electron impact on monomethylquinolines. The loss of HCN has been observed in the mass spectra of oxygenated quinolines by Clugston and MacLean (1966) and in the mass spectra of alkylisoquinolines by Marx and Djerassi (1968).

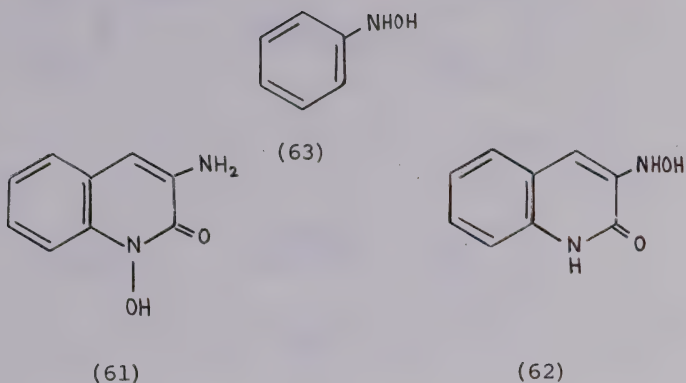
#### Mass Spectrometry of Hydroxamic Acids

Although cyclic hydroxamic acids are o-hydroxy N-oxides, no detailed study of the effect of electron impact had been reported until the present study was undertaken. Isolated references to specific hydroxamic acids are to be found in the literature. Bryce and Maxwell (1965) observed that 6,7-dimethoxy-2-hydroxy-3-oxoisoquinoline (60) can tautomerize to the corresponding N-oxide, thus behaving like the N-oxides and giving rise to an  $(M-16)^+$  ion of 44% relative abundance.



Baxter and Swan (1967) found that the base peak in the mass spectrum of what was suspected to be 3-amino-1-hydroxy-2(1H)-quinolone (61) was due to the loss of an





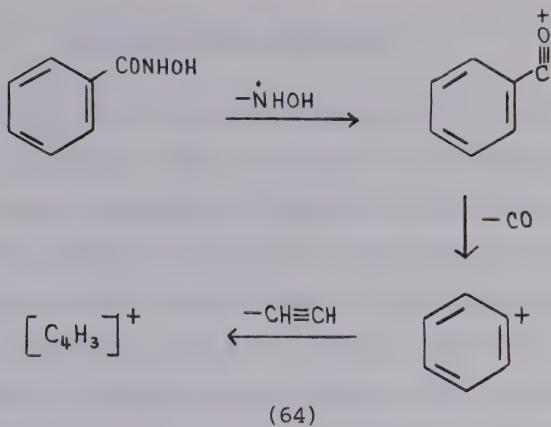
oxygen atom. Coutts et al (1969) have postulated that Baxter and Swan's compound was not 3-amino-1-hydroxy-2(1H)-quinolone but the isomer, 3-hydroxylamino-2(1H)-quinolone (62). This structure is still consistent with the observation that the base peak in the mass spectrum was the  $(\text{M}-16)^+$  ion. Phenylhydroxylamine (63) and other aromatic hydroxylamines give strong  $(\text{M}-16)^+$  ions and weak  $(\text{M}-2)^+$  ions on electron impact (Coutts and Mukherjee, 1969).

An  $(\text{M}-16)^+$  ion was observed by Abramovitch, Coutts and Pound (1967) in the mass spectrum of 3-(o-acetamidophenyl)-1-hydroxy-2(1H)-quinolone (9a).

More recently, Bowie, Hearn and Ward (1969) have reported that simple acyclic hydroxamic acids undergo a series of diagnostic fragmentation processes upon electron impact (64). A similar fragmentation process is observed for aromatic esters ( $\text{ArCOOR}$ ), ketones ( $\text{ArCOR}$ ) and







amides ( $\text{ArCONHR}$ ) (Budzikiewicz, Djerassi and Williams, 1967, 1967a, 1967b).

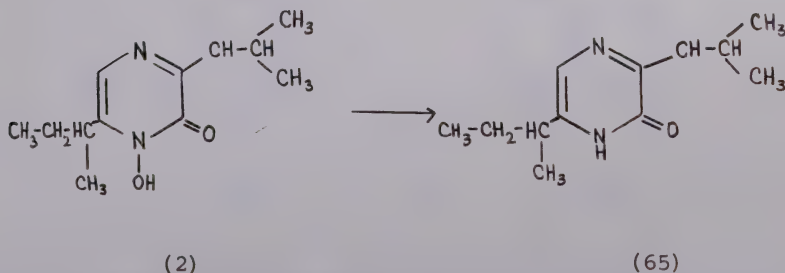


## Antibacterial Properties

In 1943, White and Hill reported that a strain of Aspergillus flavus, grown on certain liquid media yielded filtrates that possessed antibacterial activity against certain Gram negative as well as Gram positive bacteria. This was the second reported case of such behavior by a mold, the first being that of Fleming (1929) who found that a strain of Penicillium notatum formed a product that was inhibitory but not markedly lethal to certain Gram positive bacteria.

### Importance of -N(OH)CO- Grouping for Activity

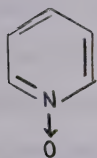
That the antibacterial activity of aspergillic acid is due, in part at least, to the hydroxylated nitrogen atom is shown by the fact that mild reduction of aspergillic acid (2) gave deoxyaspergillic acid (65), a product which had no antibacterial properties (Dutcher, 1947; Shaw, 1949).



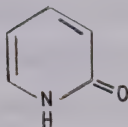
Lott and Shaw (1949) tested some pyridine and quinoline derivatives for their antimicrobial activity and



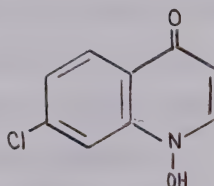
found that the common structural feature of those with antibacterial properties was the cyclic hydroxamic acid grouping. A number of structurally related compounds were tested, among them being pyridine N-oxide (66) and 2-pyridone (67). These were inactive. Furthermore, vinyllogs of hydroxamic acids such as 7-chloro-1-hydroxy-4(1H)-quinolone (68) and 1-hydroxy-4(1H)-pyridone (69) which have an acidity comparable to their isomeric 1-hydroxy-2(1H)-pyridone (15) and 2-quinolone (21) derivatives, but unlike the latter, do not chelate with metallic ions, are inactive in vitro antimicrobial agents.



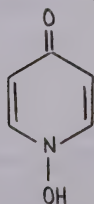
(66)



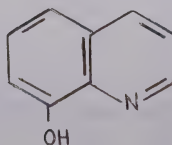
(67)



(68)



(69)



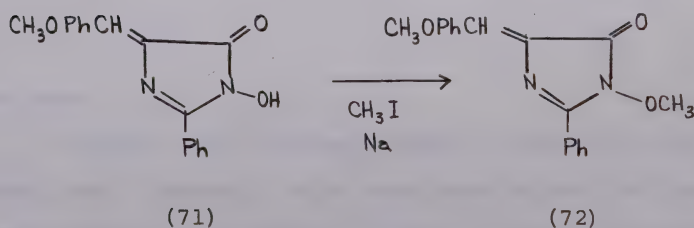
(70)

Oxine (70) is known to exhibit antibacterial properties only when ferric ions are present in the medium. This has been discussed recently in detail by Albert (1968). Goth (1945), however, has observed that the addition of



ferric ions to aspergillic acid diminishes the in vitro activity of the antibiotic. It seems probable, therefore, that hydroxamic acids are antibacterial due to their ability to combine with and deprive micro-organisms of metabolically essential metals.

Kochetkov et al (1960) found that imidazolinolone derivatives of type (71) possess high bacteriostatic action in vitro against Gram positive and Gram negative bacteria including (in some cases) Mycobacterium tuberculosis. The activity of this entirely new group of antimicrobial agents is evidently associated with the presence of the -CONOH- grouping since a change in this part of the molecule results in complete loss of activity. For example, 3-hydroxy-5-p-methoxybenzylidene-2-phenyl-1-imidazolin-4-one (71) on treatment with methyl iodide and sodium yields 3-methoxy-5-p-methoxybenzylidene-2-phenyl-1-imidazolin-4-one (72) which has no activity.

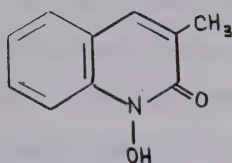


The antibacterial activity of some quinolones have been investigated by Coutts et al (1965). It is signif-

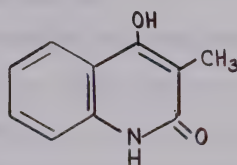




icant to note that all compounds which do show activity possess the hydroxamic acid group. The most active, 1-hydroxy-3-methyl-2(1H)-quinolone (73), can be contrasted with the closely related lactam 4-hydroxy-3-methyl-2(1H)-quinolone (74) which has no activity against S. aureus or E. coli. Ethyl 1-hydroxy-2(1H)-quinolone-3-carboxylate

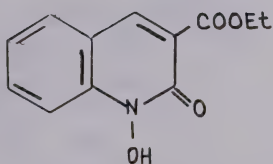


(73)

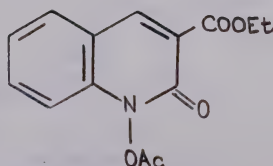


(74)

(75) exhibited activity whereas the 1-acetoxy derivative (76)



(75)

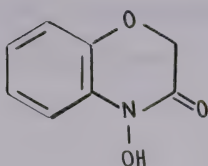


(76)

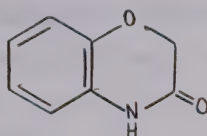
had none.

Honkanen and Virtanen (1960) and Wheeler (1962) found that hydroxamic acids were more effective than lactams against E. coli and S. aureus. Thus, 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (77) was twice as effective as the corresponding lactam (78).





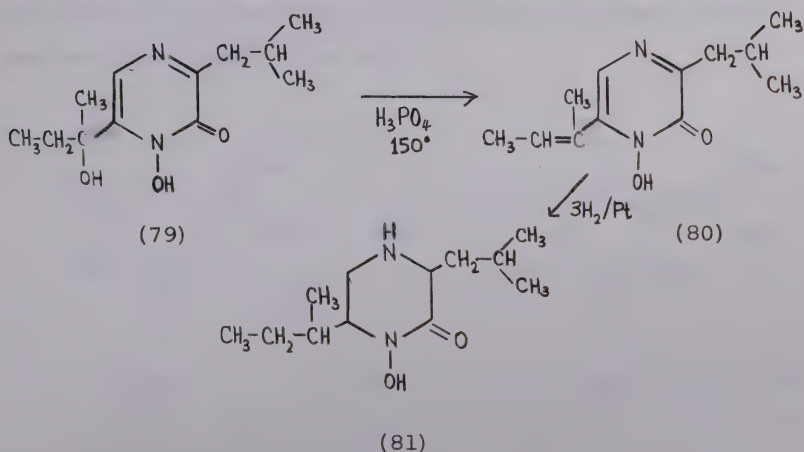
(77)



(78)

### Hydroxamic Acids Related to Aspergillic Acid

Dutcher (1958) isolated and prepared derivatives of aspergillic acid which were subjected to antibacterial testing. Hydroxyaspergillic acid (79) was isolated from Aspergillus flavus and was subjected to dehydration and reduction procedures giving dehydroaspergillic acid (80) and tetrahydroaspergillic acid (81). Only dehydroaspergillic acid exhibited greater activity against E. coli

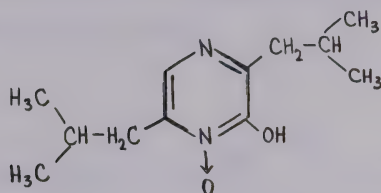


than aspergillic acid.

MacDonald et al (1964) report the isolation of 3,6-diisobutyl-2-hydroxypyrazine 1-oxide (neohydroxyaspergil-



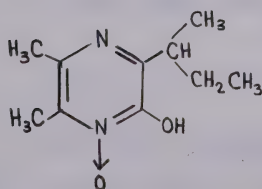
lic acid) (82) from Aspergillus sclerotiorum. The activity of this acid is close to that of aspergillic acid, which



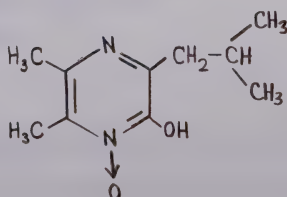
(82)

is not surprising due to the structural resemblance.

Safir and Williams in 1952 prepared cyclic hydroxamic acid derivatives of pyrazine. Both 3-secbutyl-2-hydroxy-5,6-dimethylpyrazine 1-oxide (83) and 3-isobutyl-2-hydroxy-5,6-dimethylpyrazine 1-oxide (84) were less active than aspergillic acid.



(83)



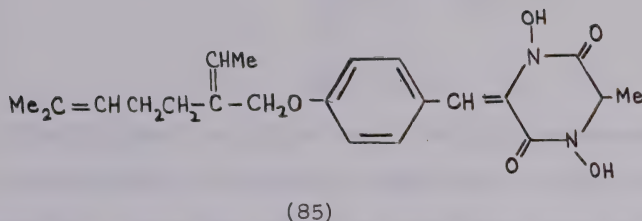
(84)

#### Other Naturally Occurring Hydroxamic Acids

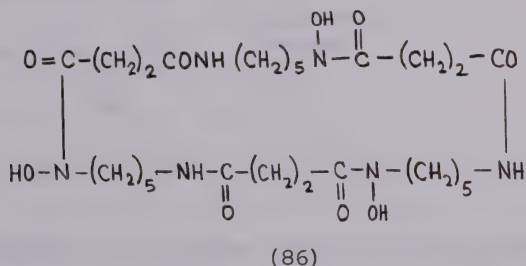
Besides aspergillic acid there are a number of naturally occurring hydroxamic acids which have antibac-



terial activity. Mycelianamide (85) which completely inhibits the in vitro growth of a number of Gram positive organisms is a product of Penicillium griseofulvum metabolism (Birch et al, 1956).



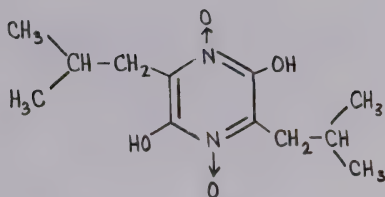
Norcardamin (86), is an antibiotic substance produced by a Norcardia species isolated from old bee honeycombs (Stoll et al, 1951; Merck Index, 1960; Keller-Schierlein and Prelog, 1961).



The yeast, Candida pulcherrima produces a red pigment, pulcherrimin. This complex is converted by means of acidification of the morpholinium salt to pulcherrimic acid which has the structure 2,5-diisobutyl-3,6-dihydroxypyrazine 1,4-dioxide (87) (MacDonald, 1963).

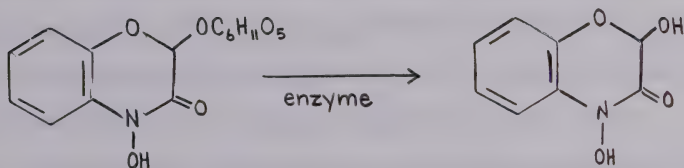






(87)

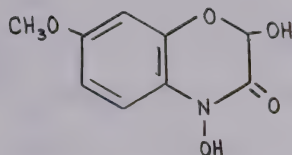
3,4-Dihydro-2,4-dihydroxy-3-oxo-2H-1,4-benzoxazine (88) was obtained by enzymatic hydrolysis of the naturally occurring glucoside after rye seedlings were crushed (Virtanen and Hietala, 1960). This benzoxazine was also isolated from maize by Tipton *et al* (1967).



(88)

Riemann and Byerrum (1964) have reported the isolation of dimboa from corn seedling and seedlings of other grasses. This compound which is a simple derivative of (88), exhibits antimetabolic activity. Chemically dimboa is 3,4-dihydro-2,4-dihydroxy-7-methoxy-3-oxo-2H-1,4-benzoxazine (89) (Hamilton *et al*, 1962; Tipton *et al*, 1967).



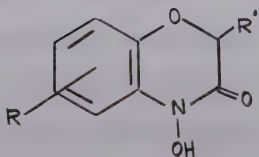


(89)

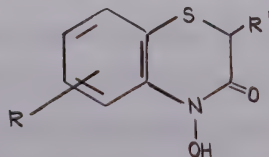
### Hydroxamic Acid Derivatives of Pyridine and Quinoline and Related Compounds

N-hydroxy derivatives of 2(1H)-pyridone and 2(1H)-quinolone have been tested for their antibacterial activity (Shaw, 1949; Newbold and Spring, 1948; Cunningham *et al*, 1949; Coutts *et al*, 1964). The activity is influenced by the substitution on the nucleus. In the quinolone series Coutts *et al* (1965) found that an alkyl group is preferred at position 3- and the most active compound was 1-hydroxy-3-methyl-2(1H)-quinolone (73).

Coutts *et al* (1964) and Coutts and Hindmarsh (1966) extended antibacterial testing to other systems; benzoxazines (90), benzothiazines (91), quinoxalines (92), and quinazolines (93) were examined. *In vitro* antibacterial

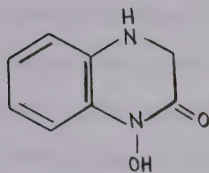


(90)

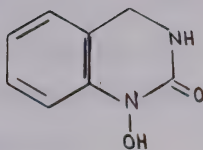


(91)





(92)



(93)

activities showed that no compound had a broader spectrum of activity than the quinoline, 1-hydroxy-2(1H)-quinolone (21).

Shaw et al (1950) previously studied the in vitro activity of other sulfur-containing hydroxamic acids. 2-Bromopyridines were converted to the N-oxide with perbenzoic acid or peracetic acid and treatment with sodium sulfide or sodium hydrosulfide under mild conditions gave the thiohydroxamic acids (see previous discussion). The pyridinethiones were compared to N-hydroxy-2(1H)-pyridones and aspergillic acid. They were found to be more active in every instance against S. aureus and Klebsiella pneumoniae.

There are numerous examples in the literature of acyclic hydroxamic acids with reported antibacterial and antifungal activities. As the present study is limited to cyclic hydroxamic acids the acyclic hydroxamic acids will not be discussed. The latter compounds have been reviewed recently (Coutts, 1967).



### The Present Investigation

1. Because there has been no detailed study on the effect of electron impact on cyclic hydroxamic acids it was important to find out whether mass spectrometry would serve as a method of characterizing these compounds.
2. Because 3-methyl-2(1H)-quinolone has been reported to have antibacterial activity it was desirable to prepare other hydroxamic acids that are chemically related and to investigate the effect of substitution on antimicrobial activity.

The present work therefore deals with the preparation and properties of new cyclic hydroxamic acids.





PART I  
DISCUSSION



### Preparation of Alkyquinolines

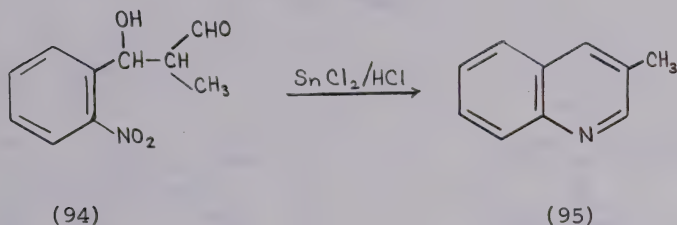
It has been reported in the literature (Coutts, Pitkethly and Wibberley, 1965) that 1-hydroxy-3-methyl-2(1H)-quinolone (73) is an efficient antibacterial agent. It was desirous, therefore, to prepare other hydroxamic acids, chemically related to this quinolone, to investigate the effect of substitution on the antimicrobial activity of these compounds. A sample of 1-hydroxy-3-methyl-2(1H)-quinolone was also required for comparison purposes. The activity of each new hydroxamic acid was to be compared with that of the above mentioned quinolone.

3-Methylquinoline was a required intermediate. A general method does not exist for the preparation in good yields of 3-alkylquinolines, although various methods have been reported. The classical Friedlaender synthesis is generally applicable but is limited by the difficulty in obtaining aromatic o-amino-carbonyl compounds, such as o-aminobenzaldehyde, as the starting materials (Utermohlen, 1943). 3-Methylquinoline has been prepared recently (Kawazoe and Tachibana, 1967) using the Friedlaender synthesis by reducing o-nitrobenzaldehyde and condensing the resulting amine with propionic aldehyde.

The preparation of  $\beta$ -o-nitrophenyl- $\alpha$ -methyl-hydracrylaldehyde (94) from o-nitrobenzaldehyde and prop-



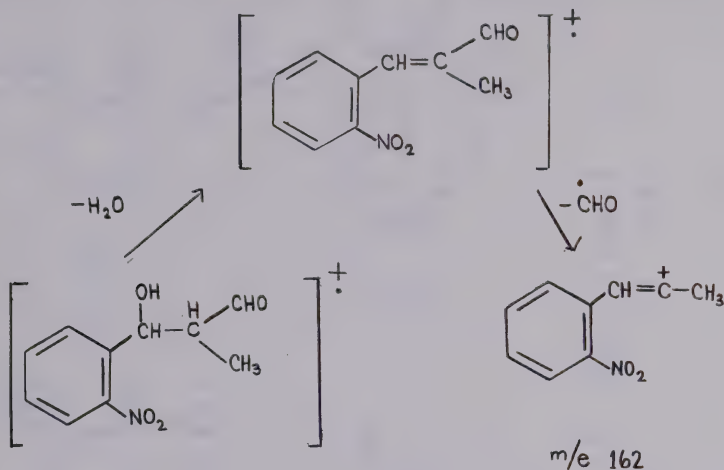
ionaldehyde was reported by Willimott and Simpson in 1926. They observed that 3-methylquinoline (95) could be obtained in an 80% yield by reductive cyclization of this aldehyde with stannous chloride and concentrated hydrochloric acid. In the present study, a procedure



similar to that described by Willimott and Simpson for the preparation of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracryl-aldehyde was used. A viscous oil was obtained which did not crystallize. Its infrared spectrum showed peaks which indicated the presence of hydroxyl, carbonyl and nitro groups in the molecule. The presence of an aldehyde group was further substantiated by the formation of a 2,4-dinitrophenylhydrazine derivative. Thin layer chromatography of the oil, using a solvent system of benzene and methanol, indicated that the oil had one major component. The oil was thus subjected to mass spectrometry and it appeared from the mass spectrum that the oil was the required compound. The molecular ion was not present in the spectrum. Ions of m/e 162, 152 and abundant ions of m/e 122, 121, 93 and 65 were observed, however.



The fragment ion of  $m/e$  162 is possibly due to the loss of water and the aldehyde portion of the compound under study:



The loss of water from the molecular ion is known to occur in compounds containing a hydroxyl group alpha to the benzene ring. Adrenaline and noradrenaline, for example, lose 18 mass units (Reisch et al, 1968). The spectra of cinnamaldehyde and  $\alpha$ -methylcinnamaldehyde show abundant  $(M-29)^+$  ions. According to Brittain, Kelly and Mead (1969) the loss of 29 mass units does not occur by simple cleavage alpha to the double bond but rather is the result of the expulsion of a hydrogen radical firstly and then a CO molecule. The other ions may be accounted for by Scheme 1.  $\beta$ -cleavage giving the ion  $m/e$  152 is a known process for compounds possessing a hydroxyl group alpha to the benzene ring (Reisch et al,









1968). Although there were no metastable ions present in the spectrum to support the direct loss of nitric oxide, it is well known that nitro compounds do undergo rearrangement and lose nitric oxide when subjected to electron impact (Budzikiewicz, Djerassi and Williams, 1967d).

The period of almost two weeks that was required for the preparation of the aldehyde (94) by the method described by Willimott and Simpson, was time consuming and an alternate procedure was sought. A 3% aqueous barium hydroxide solution was used as the basic catalyst instead of piperidine and after a period of twenty two hours (at room temperature) a reddish oil was obtained by extracting the reaction mixture. Thin layer chromatography of this oil revealed that it contained at least four components. The leading component was collected from a preparative silica gel plate and identified as o-nitrobenzaldehyde. Because this oil was impure further investigations on it were not carried out.

A sample of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde was treated with stannous chloride and concentrated hydrochloric acid according to the procedure described by Willimott and Simpson (1926). A very poor yield of a basic product was obtained as an oil. This oil was identified by the infrared spectrum as being 3-methylquinoline but the yield was so low that it was not purified.



Coutts (1969) has reported the preparation of 3-cyano-1-hydroxy-2(1H)-quinolone and ethyl 1-hydroxy-2(1H)-quinolone-3-carboxylate (75) by reductive cyclization of a suitable o-nitrophenyl-ester with sodium borohydride in the presence of palladium-charcoal. Because of these results attempts were made to prepare the corresponding acid of  $\beta$ -o-nitrophenyl- $\alpha$ -methyl-hydracrylaldehyde. Attempts to oxidize the aldehyde (94) with potassium permanganate in glacial acetic acid resulted in recovery of starting material when the reaction was done at room temperature and when the reaction mixture was boiled under reflux for six and one-half hours. Attempted oxidation with silver nitrate, in a basic medium, again resulted in recovery of the starting material and a small amount of a solid identified as o-nitrobenzoic acid. Because of the difficulty in obtaining the acid, the aldehyde was reduced using sodium borohydride and palladium-charcoal. An oil was isolated from the reaction mixture and its infrared spectrum did not show any nitro-absorption. The oil was separated into acidic, basic and neutral components and each was subjected to thin layer chromatography. Figure 1 shows that a complex mixture had been obtained. The neutral portion was composed of nine components, the acidic portion contained eight components and the basic layer contained six components. The reduction was repeated at a lower temperature but a complex mix-



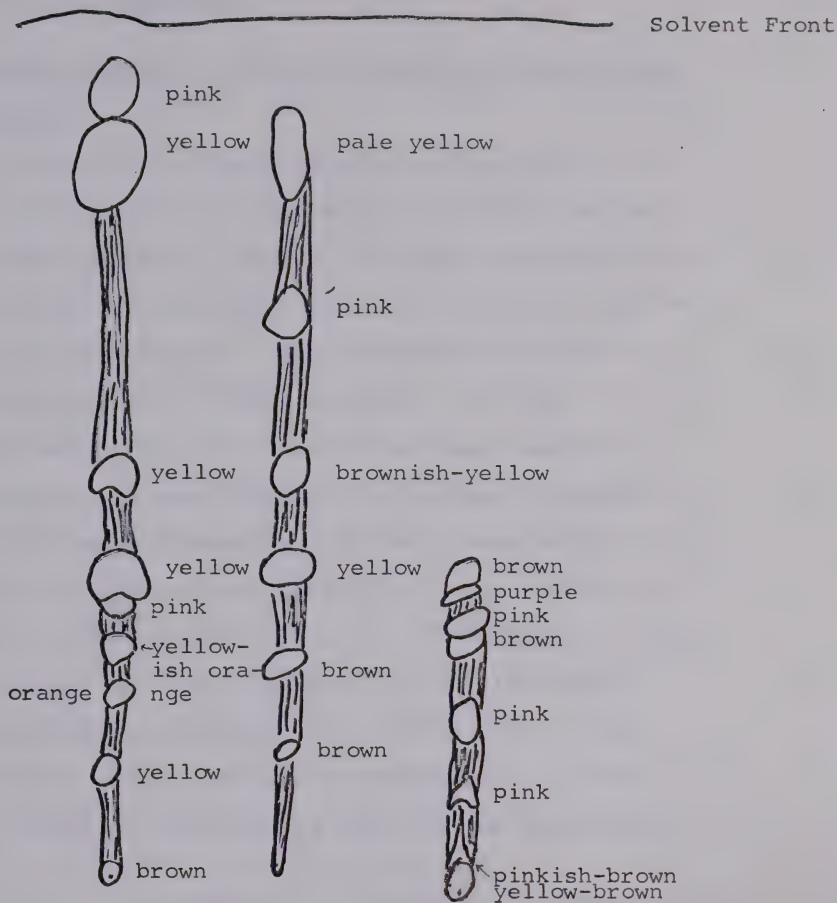


Figure 1: A thin layer chromatogram of (1) neutral, (2) basic and (3) acidic portions obtained by reduction of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde with sodium borohydride and palladium-charcoal. Adsorbant: Silica Gel G (Merck). Solvent system: Benzene: Methanol (10:1.5)





ture was still obtained.

Gas chromatography of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde

Initially gas chromatography was carried out on a carbowax column. A programmed run did not give reproducible results. However, two major components with widely differing retention times were collected when an isothermal run was done. The infrared spectra of the two components were almost identical; carbonyl absorption and nitro-group absorption were observed in both spectra. A mass spectrum of the first component showed the heaviest abundant ion had a mass of  $m/e$  263 (42%) but the presence of weak ions at  $m/e$  281 and 336 suggested impurity and a meaningful interpretation could not be made. An isothermal run of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde on an SE 30 (10%) column showed the presence of three components with the following retention times at the three column temperatures used:

	150°	200°	225°
Component 1	1.14 min	0.94 min	0.63 min
Component 2	2.84 min	2.50 min	1.60 min
Component 3	11.00 min	8.00 min	3.30 min

Components 2 and 3 were the major ones. Component 2 was collected and identified as o-nitrobenzaldehyde. An infrared spectrum of the third component contained a



carbonyl absorption band at  $1685\text{ cm}^{-1}$  and nitro absorption bands at  $1338$  and  $1520\text{ cm}^{-1}$ . The mass spectrum of this oil was non-informative. An ion of  $m/e$  162 was the heaviest ion observed but this must be a fragment ion because compounds that contain one nitrogen atom should have an odd mass. No definite conclusions as to the identity of this oil could be made. Because o-nitrobenzaldehyde was one of the major components obtained on gas chromatography a physical mixture of o-nitrobenzaldehyde and propionaldehyde was prepared. The infrared spectrum differed with that of the product obtained by Willimott and Simpsons' procedure. Gas chromatography of  $\beta$  -o-nitrophenyl- $\alpha$  -methylhydracrylaldehyde was repeated at  $125^\circ$  with simultaneous injections of o-nitrobenzaldehyde and propionaldehyde. Peak augmentation occurred for components 1 and 2 identifying them as propionaldehyde and o-nitrobenzaldehyde respectively. This would indicate that the aldehyde (94) is not too stable when heated.

$\beta$  -o-Nitrophenyl- $\alpha$  -methylacrylic acid was synthesized from o-nitrobenzaldehyde and propionic anhydride according to the procedure of Cunningham et al (1949). The ethyl ester was prepared and reduced with sodium borohydride and palladium-charcoal. This treatment yielded only a small amount of 1-hydroxy-3-methyl-2(1H)-quinolone (73) (see discussion on reductive cyclizations by means of sodium borohydride and palladium-



charcoal, page 78).

Failure to obtain 3-methylquinoline in good yield from  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde led to an investigation of other methods of synthesis. Coutts, Pitkethly and Wibberley (1965) reported a preparation of 3-methylquinoline by the decarboxylation of 3-methylquinoline-4-carboxylic acid. The latter compound was synthesized in this present study by the method of Ornstein (1907) using isatin and propionaldoxime, and decarboxylation of the product produced an oil in a very low yield which was not adequate for the purpose of this study. Finally, a sufficient quantity of the desired alkylquinoline was obtained by a procedure described by Manske (1942). Aniline and methacrylaldehyde were heated at  $140^{\circ}$  with ferrous sulphate, o-nitrophenol and concentrated sulfuric acid and a mixture of aniline and 3-methylquinoline were obtained by steam distillation of the basified reaction mixture. 3-Methylquinoline was finally obtained as a light yellow oil by vacuum distillation.

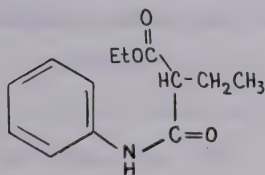
#### Attempted preparation of 3-ethylquinoline

A synthesis similar to that described for 3-methylquinoline-4-carboxylic acid has been reported by Mulert (1906) for the preparation of 3-ethylquinoline-4-carboxylic acid. Mulerts' procedure was repeated. 3-Ethylquinoline-4-carboxylic acid was obtained in such

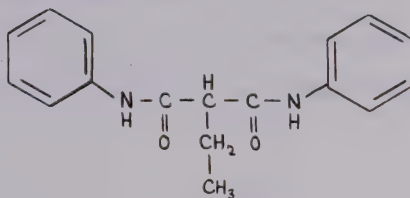


a poor yield, however, that an alternative approach was attempted.

Chattaway and Olmstead (1910) obtained ethyl malonanilate by gently heating a mixture of aniline and ethyl malonate. Ethyl ethylmalonanilate (96) and the by-product ethylmalonanilide (97) were obtained, in this present study, from aniline and diethyl ethylmalonate by a similar procedure. The crude sample of



(96)



(97)

ethyl ethylmalonanilate was purified by column chromatography. Microanalysis, nuclear magnetic resonance (NMR) and infrared spectroscopy were used to verify that the required compound had been obtained because although Draper and MacLean (1968) report the use of this compound they do not describe any of the physical characteristics. Ethyl ethylmalonanilate was then treated with thionyl chloride. The oily product obtained from the reaction still showed ester carbonyl absorption ( $1730\text{ cm}^{-1}$ ) in the infrared spectrum. Treatment with phosphorous oxychloride (Draper and MacLean,

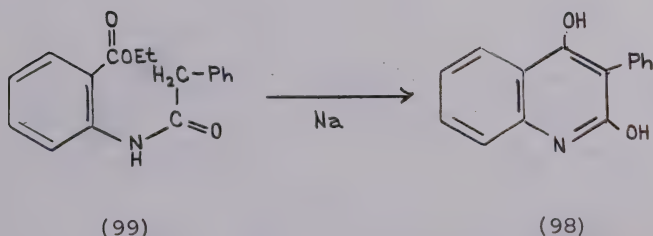




1968) for ten hours and for sixty five hours yielded an oil which similarly showed ester absorption in the infrared spectrum. Only a small amount of basic material was extractable from this oil with 10% hydrochloric acid. The cyclized product, 2,4-dichloro-3-ethylquinoline, reported by Draper and MacLean, was not obtained. An attempted reaction with phosphorous pentachloride was also unsuccessful.

#### Attempted preparation of 3-phenylquinoline

deDiesbach et al, (1951) were able to obtain 2,4-dihydroxy-3-phenylquinoline (98) from ethyl N-phenacetylanthranilate (99). Ethyl N-phenacetylanthranilate was

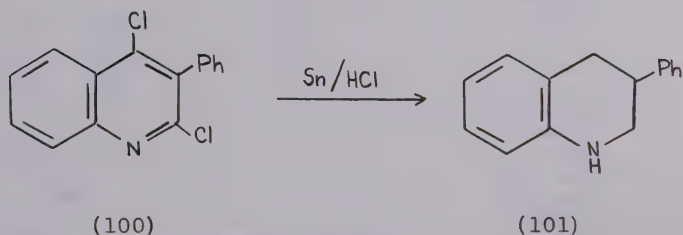


successfully obtained in this study by stirring phenyl acetyl chloride and ethyl anthranilate in a sodium hydroxide solution. When phenyl acetyl chloride was stirred in an ether solution according to a procedure described by deDiesbach et al (1951), the only product isolated was the hydrochloride salt of ethyl anthranilate. Ethyl N-phenacetylanthranilate (99) was



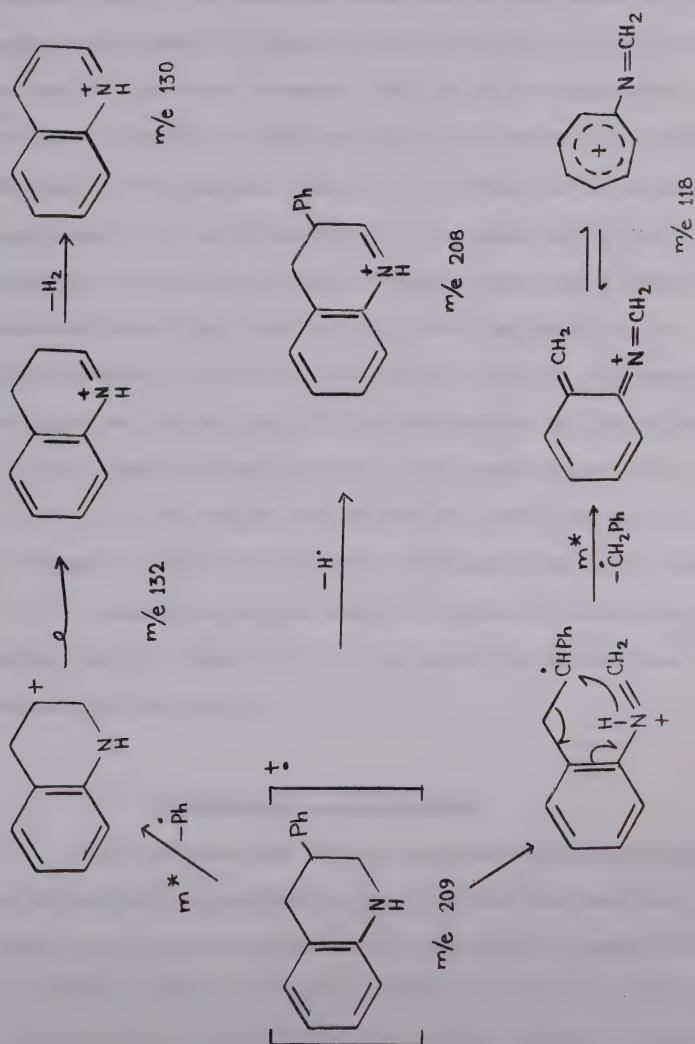
converted into 2,4-dihydroxy-3-phenylquinoline by boiling under reflux with metallic sodium and the 2,4-dichloro-derivative (100) was readily obtained, in good yield, by treating the 2,4-dihydroxy compound with phosphorous oxychloride.

Treatment of 2,4-dichloro-3-phenylquinoline with tin and hydrochloric acid gave a yellow solid which melted at  $86-88^{\circ}$ . The infrared spectrum showed an absorption peak at  $3400\text{ cm}^{-1}$  which was thought to be due to N-H stretching. A mass spectrum revealed that the parent ion was  $m/e$  209 which would indicate that 3-phenyl-1,2,3,4-tetrahydroquinoline (101) had been formed. Booth and Crisp (1964) have reported the syn-



thesis of 3-phenyl-1,2,3,4-tetrahydroquinoline by the hydrogenation of 3-phenylquinoline, a procedure similar to that reported by Hubner in 1906. The possibility that 3-phenyl-1,2,3,4-tetrahydroquinoline had been obtained was substantiated by comparing the mass spectrum with that of 3-methyl-1,2,3,4-tetrahydroquinoline. Draper and MacLean (1968a) found that the mass spectrum





Scheme 2



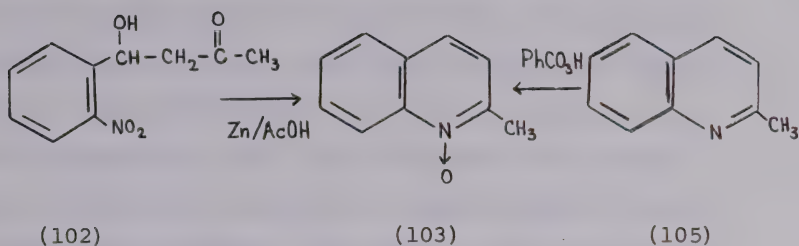
of 3-methyl-1,2,3,4-tetrahydroquinoline had abundant  $(M-1)^+$  and  $(M-29)^+$  ions giving ions of  $m/e$  146 and 118 respectively. A similar observation was made in the mass spectrum of 3-phenyl-1,2,3,4-tetrahydroquinoline. An  $(M-1)^+$  ion was present (18% relative abundance) and an ion of  $m/e$  118 (58% relative abundance) was also present. The latter ion can be formed in a manner analogous to the formation of the same ion from 3-methyl-1,2,3,4-tetrahydroquinoline and its formation is substantiated by a suitably placed metastable ion. Furthermore, the loss of a phenyl radical (Scheme 2) to give an ion of  $m/e$  132 is analogous to the behaviour of the 3-methyl derivative. Both spectra exhibit an ion at  $m/e$  130 which can be due to the loss of two hydrogens from ion  $m/e$  132. The spectrum of 3-phenyl-1,2,3,4-tetrahydroquinoline is virtually identical with that of 3-methyl-1,2,3,4-tetrahydroquinoline at masses below  $m/e$  132.

### Preparation of N-oxides

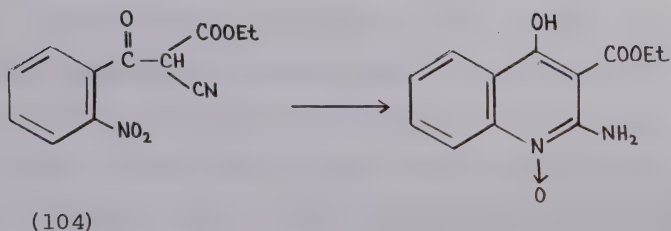
The preparation of cyclic N-oxides can be achieved by oxidative methods or by cyclization methods. Reductive cyclizations can be carried out using zinc and acetic acid. A typical example is the reduction of  $\beta$ -hydroxy- $\beta$ -(o-nitrophenyl)ethyl methyl ketone (102) to 2-methylquinoline 1-oxide (103). Many derivatives of quinoline 1-oxide have been synthesized by this







method (Ochiai, 1967). Other reductive cyclization methods have been reported. Coutts and Wibberley (1962), for example, obtained an N-oxide by heating an ethanolic solution of ethyl  $\alpha$ -cyano- $\alpha$ -o-nitrobenzoylacetate (104) with palladium-charcoal using cyclohexene as the hydrogen donor. Because N-oxides of the quinoline series



can be prepared by direct oxidation, reductive cyclization methods are little used.

#### Preparation of N-oxides by oxidation

In 1925, Meisenheimer and Stotz carried out the oxidation of quinaldine (105) with perbenzoic acid in benzene solution and obtained a product identical with



the substance derived from the reduction of  $\beta$ -hydroxy- $\beta$ -(o-nitrophenyl)ethyl methyl ketone by the action of zinc and acetic acid as described by Heller and Sourlis (1908). Soon after this, Meisenheimer (1926), by employing perbenzoic acid, oxidized other ring systems such as quinoline, pyridine and isoquinoline to their corresponding N-oxides. This method often necessitated isolation and purification of the N-oxide as its picrate.

Böhme in 1937 suggested the use of monoperphthalic acid in place of perbenzoic acid as the oxidation agent. The N-oxide would then be expected to separate as its crystalline phthalate, thereby facilitating purification.

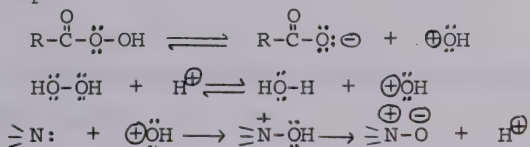
In 1938, Clemo and McIlwain found that the action of hydrogen peroxide or benzoyl peroxide on phenazine in a neutral medium resulted in recovery of the starting material, whereas the use of Caro's acid or better still 5% hydrogen peroxide in glacial acetic acid resulted in the formation of phenazine N,N'-dioxide in good yields. Later, in 1945, Ochiai and Zai-Ren found that this peracetic acid method could be used to oxidize pyridine and quinoline to their N-oxides.

If an amine has sufficient basicity for N-oxidation and is comparatively stable to side reactions, then oxidation by means of hydrogen peroxide and acetic acid is the simplest and most convenient method. Acetic acid is a good solvent for most amines because reaction conditions can be made stronger by heating and the solvent is suitable



for treating a large quantity (Ochiai, 1967a). Synthesis with peracids usually requires ether as the solvent and large scale preparations would necessitate carrying out the reaction at a low temperature.

N-Oxidation using a percarboxylic acid or hydrogen peroxide in glacial acetic acid is the result of bond formation between a lone pair of electrons of the amine nitrogen and electron-deficient hydroxyl groups polarized to produce electrophilic activity (Ochiai, 1967b). The progress of the reaction therefore, should depend mainly on the basicity of the nitrogen atom and on the ability of the oxidizing agent to form a positively charged hydroxyl group.



Successful oxidations were carried out in this present study by reacting quinolines with 30% aqueous hydrogen peroxide and glacial acetic acid. The following products were obtained in the yields stated: quinoline 1-oxide (59%), isoquinoline 2-oxide (39%) and 4-methylquinoline 1-oxide (83%). The higher yield of 4-methylquinoline 1-oxide may be due to the positive inductive effect of the methyl group in the 4-position and also due to the fact that a higher melting N-oxide is easier to isolate. In a similar manner, the following N-oxides were obtained, in the yields stated, from the appropriate quinoline: 5-



nitroquinoline 1-oxide (76%), 6-nitroquinoline 1-oxide (49%), 3-methylquinoline 1-oxide (34%), 3,6-dimethylquinoline 1-oxide (66%), 3,7-dimethylquinoline 1-oxide (63%), 6,7-dimethylquinoline 1-oxide (48%).

The N-oxides of 3,8-dimethylquinoline, 6,8-dimethylquinoline and 7,8-dimethylquinoline were not formed when attempts were made to oxidize the quinolines with hydrogen peroxide and glacial acetic acid. Brown oils were isolated and the infrared spectra of these oils did not show any N-O absorption peaks. The presence of an 8-methyl-substituent may cause a steric hindrance and prevent an attack by the oxidizing agent on the nitrogen atom.

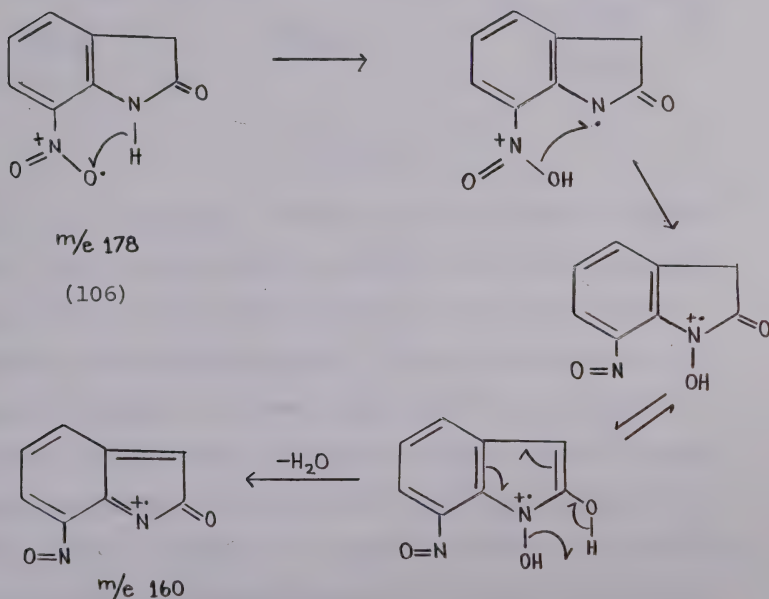
#### Treatment of 8-nitroquinoline with hydrogen peroxide and glacial acetic acid

Similar oxidative treatment of 8-nitroquinoline did not give the desired N-oxide. A large recovery of the starting material was obtained as well as a red solid which melted at 256-257°. An examination of the infrared spectrum of this solid showed the presence of an NH group ( $3200\text{ cm}^{-1}$ ), a carbonyl ( $1730\text{ cm}^{-1}$ ) and a nitro group ( $1515, 1350\text{ cm}^{-1}$ ). The compound was subjected to mass spectrometry. The  $\text{M}^+$  peak was located at  $m/e$  178 and an accurate mass determination indicated a molecular formula of  $\text{C}_8\text{H}_6\text{N}_2\text{O}_3$ . This evidence suggested that the product obtained was 7-nitrooxindole (106). An abundant





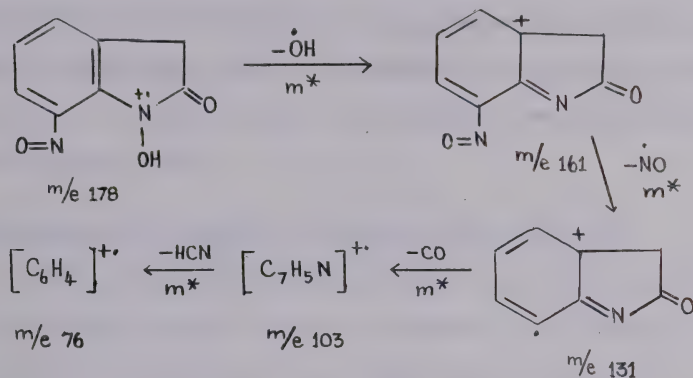
ion in the mass spectrum of this product appeared at  $m/e$  160 and the presence of a metastable ion at  $m/e$  143.8 indicated that this ion was due to the direct loss of 18 mass units (i.e. water) from the molecular ion. A mass spectrum of oxindole was obtained and the loss of water was not observed. Therefore, it is possible that the nitro-group in the 7-position participates in this loss:



A similar involvement of a nitro- group is reported for the loss of a CO molecule from 1-nitronaphthalene, involving the peri-hydrogen (Budzikiewicz, Djerassi and Williams, 1967e). Other abundant ions in the mass spec-



trum were found at 178, 161, 131, 103 and 76 and may be accounted for by the following scheme (3):



Scheme 3

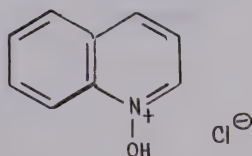
The parent compound, oxindole does not lose an OH radical thus the loss of this radical from this compound may be due to the participation of the nitro-group. Oxindole does however expel a CO and HCN molecule on electron impact. An NMR spectrum of the product obtained in this reaction showed a broad aromatic proton region between  $\tau$  0.02 to  $\tau$  2.9 and the signal integrated for 3 protons. A 2-proton singlet was found at  $\tau$  6.7 and a 1-proton singlet at  $\tau$  6.32 which were tentatively assigned to methylene and  $\text{NH}$  protons respectively. The unusual position of the  $\text{NH}$  proton may be due to a shielding effect by the nitro group (Bovey, 1969).



A similar oxidation of quinoline-8-carboxylic acid (Ochiai et al, 1960) is reported to give 3-hydroxyquinoline-8-carboxylic acid. A nitro group or a carboxylic acid group in the 8-position may prevent N-oxidation by steric hindrance, thus not allowing the oxidizing species to attack at the nitrogen, or decrease the basicity of the nitrogen atom.

### Preparation of 3- and 4-nitroquinoline N-oxides

The products obtained by electrophilic substitution on quinoline 1-oxide depend on whether the compound is acting as a free base or as a derivative such as 1-hydroxyquinolinium chloride (107) (Palmer, 1967). Nitration of quinoline 1-oxide with nitric acid and sul-

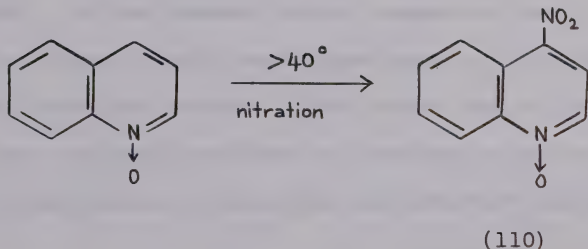
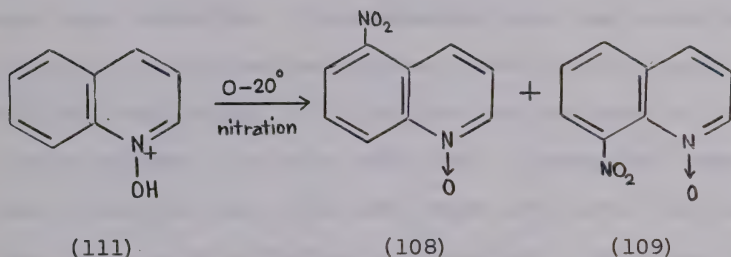


(107)

furic acids at 0-20° gives a mixture of 5- and 8-nitroquinoline 1-oxides (108 and 109) and this is reminiscent of the result of nitrating quinoline itself. However, at a temperature of 80° the product of nitration with the same mixture of acids is the 4-nitro derivative (110). It seems that this reaction occurs as the



free N-oxide rather than as a protonated species. Since basic strength generally increases as the temperature is lowered (Palmer, 1967), it is possible that low temperature nitration involves the formation of a 1-hydroxyquinolinium cation (111). Ochiai and Okamoto (1950)



also observed the marked effect of changes in temperature on the nitration of quinoline 1-oxide with potassium nitrate and sulfuric acid. The effect of the presence of the N-oxide group is hardly observed at a low temperature, but above 40° the yield of the 4-nitro compound increases and this is the main product. At temperatures above 100°, deoxygenation of the N-





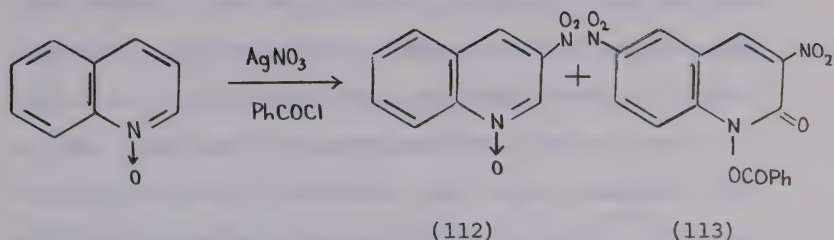
oxide occurs and the yield of 4-nitroquinoline 1-oxide decreases. In the present study 4-nitroquinoline 1-oxide was obtained in a 43% yield by heating quinoline 1-oxide with potassium nitrate and concentrated sulfuric acid.

Recently, Hamana and Nagayoshi (1966) examined the effect of changes in concentration of the sulfuric acid upon nitration. They observed that the action of a solution of potassium nitrate in 80% sulfuric acid, at room temperature, on quinoline 1-oxide gave only 4-nitroquinoline 1-oxide in 60% yield. Hamana and Nagayoshi concluded that the most important factor influencing the site of nitration of quinoline 1-oxide was not the reaction temperature but the concentration of sulfuric acid. In the present study, repeating their reaction and using their conditions gave a 53% yield of 4-nitroquinoline 1-oxide. This yield was slightly better than the yield obtained by heating quinoline 1-oxide with potassium nitrate and concentrated sulfuric acid.

Ochiai and Kaneko (1959), obtained a poor yield (32%) of 3-nitroquinoline 1-oxide (112) by adding benzoyl chloride and silver nitrate to a cooled solution of quinoline 1-oxide in chloroform. 1-Benzoyloxy-3,6-dinitrocarbostyryl (113) was also isolated in 7% yield from the reaction mixture. A repeat of their reaction conditions gave only a 13% yield of 3-nitroquinoline 1-oxide. 1-Benzoyloxy-3,6-dinitrocarbostyryl was not



obtained.



### Preparation of 4-chloroquinoline 1-oxide

A nitro group located in the position para to the N-oxide function in an aromatic N-oxide is active to nucleophilic substitution and is known to undergo easy substitution with a halogen atom. Okamoto (1951) found that 4-nitroquinoline 1-oxide when boiled in concentrated hydrochloric acid underwent substitution to form the corresponding 4-chloro compound in 95% yield. 5-Nitroquinoline 1-oxide does not react with hydrochloric acid. When the reported conditions were employed in the present study, 4-chloroquinoline 1-oxide was isolated in a 92% yield. A 95% yield of the title compound was obtained by using reaction conditions similar to those described by Ochiai (1953), in which, 4-nitroquinoline 1-oxide is added slowly and with cooling to acetyl chloride.

### Preparation of 2-chloroquinoline 1-oxide

Because 30% hydrogen peroxide and glacial acetic acid was an efficient reagent for the preparation of



other N-oxides, 2-chloroquinoline was treated in a similar manner. The only product isolated from this reaction was 2(1H)-quinolone. Reducing the reaction time from four to two hours gave the same product. Kamiya in 1961 obtained 2-chloroquinoline 1-oxide from 2-chloroquinoline by oxidizing the latter compound with hydrogen peroxide in monochloroacetic acid. The basic chloroform extract obtained on repeating this reaction was subjected to column chromatography on alumina. The first developing solvent employed was benzene. The benzene effluent contained only starting material. A mixed solvent system of benzene and methanol eluted 2(1H)-quinolone. Increasing the methanol concentration of the developing solvent did not give any further product. Using a reaction mixture of 50% hydrogen peroxide and phthalic anhydride also failed to give the N-oxide.

A literature search revealed that the presence of ring halogen atoms strongly inhibits N-oxide formation. 2-Chloroquinoline is resistant to N-oxidation by means of perbenzoic acid or acetic acid and hydrogen peroxide. The N-oxide is formed only by employing mixtures of trifluoroacetic acid and hydrogen peroxide or permaleic acid (Ochiai, 1967c).

Yamazaki et al (1966) obtained 2-chloroquinoline 1-oxide when they used maleic anhydride and 30% hydrogen peroxide to oxidize 2-chloroquinoline. Repeating



this method gave the required N-oxide in a 69% yield. Using the same reaction conditions, an attempt was made to prepare 2,4-dichloro-3-phenylquinoline 1-oxide. This was apparently not strong enough conditions as recovery of the starting material was obtained.

#### Preparation of 2-chloro-4-nitroquinoline 1-oxide

This N-oxide was prepared in 55% yield by nitrating 2-chloroquinoline 1-oxide with potassium nitrate in 80% sulfuric acid at 80° (cf. Yamazaki et al, 1968).

#### Infrared spectra of the N-oxides

It has been established that stretching of the N-O group of substituted pyridine and pyrazine N-oxides results in a strong absorption band in the 1190 to 1350  $\text{cm}^{-1}$  region of the spectrum (Shindo, 1960). The infrared spectra of the N-oxides prepared in this study were compared with the spectra of the parent quinoline. Table I gives the position of the strong absorption bands observed in the 1220-1340  $\text{cm}^{-1}$  region which were not present in the infrared spectra of the corresponding quinolines.

The infrared spectra of the oils obtained on oxidation of 3,8-dimethylquinoline, 6,8-dimethylquinoline and 7,8-dimethylquinoline did not show any strong absorption bands in the region of 1220-1340  $\text{cm}^{-1}$ .







Table I

N-oxide Absorptions of Some Quinoline 1-oxides  
in the Infrared Region of 1220-1340  $\text{cm}^{-1}$

Compound*	Wave-number ( $\text{cm}^{-1}$ )
quinoline 1-oxide (KBr)	1230, 1270
<u>iso</u> quinoline 2-oxide (KBr)	1252, 1325
4-methylquinoline 1-oxide (KBr)	1238, 1270
4-nitroquinoline 1-oxide	1300, 1330
3-nitroquinoline 1-oxide	1222, 1320
5-nitroquinoline 1-oxide	1270, 1295
6-nitroquinoline 1-oxide	1272, 1292
3-methylquinoline 1-oxide	1220
3,6-dimethylquinoline 1-oxide	1228, 1270
3,7-dimethylquinoline 1-oxide	1230, 1270, 1310
6,7-dimethylquinoline 1-oxide	1220, 1265, 1290 (m)
4-chloroquinoline 1-oxide	1225, 1250, 1302
2-chloroquinoline 1-oxide	1260, 1340
2-chloro-4-nitroquinoline 1-oxide	1310

\* All spectra were recorded as nujol mulls unless otherwise indicated.



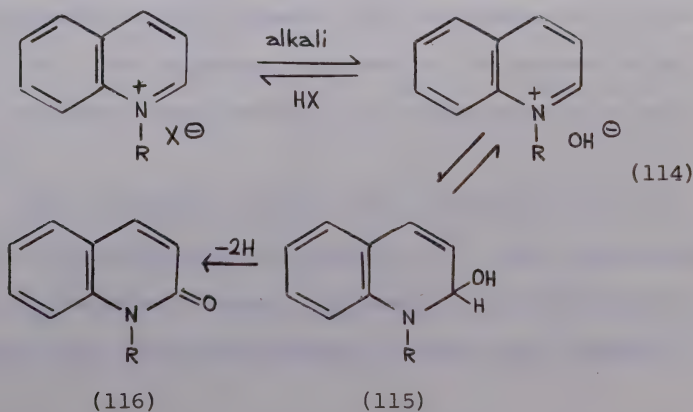
## Preparation of Hydroxamic Acids

### Oxidation by means of alkaline potassium ferricyanide

The ferricyanide ion used in oxidation procedures serves the purpose of being a complex electron-abstracting ion. Consequently, it has been used in systems favored for oxidation in this manner, that is, by extraction of an electron from an electron-rich site (Thyagarajan, 1958).

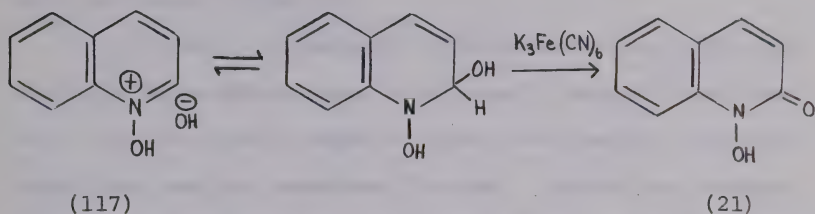


Quaternary salts of aromatic nitrogen-containing heterocycles such as N-substituted pyridinium and quinolinium salts are oxidized by alkaline ferricyanide to the N-substituted- $\alpha$ -oxo compound (Hamana and Yamazaki, 1962). This reaction is generally assumed in the quinoline series to proceed through isomerization of the corresponding quaternary hydroxide (114) to a pseudo-base (115) which is subsequently dehydrogenated by ferricyanide to a 2(lH)-quinolone (116).





It seems possible that N-oxides may be converted through (117) into their pseudo-base in an alkaline solution and might be oxidized to 1-hydroxy-2(1H)-quinolone (21) by the action of alkaline ferricyanide (Hamana and Yamazaki, 1962).



The procedure for the oxidation of some aromatic N-oxides was described by Hamana and Yamazaki in 1962, and involves adding an aqueous solution of potassium ferricyanide and a solution of potassium hydroxide, over an extended period of time, to a vigorously stirred solution of the N-oxide in water. Unreacted starting material is recovered by extracting with chloroform. Acidification of the aqueous solution gives the hydroxamic acid. The products obtained in the present study were purified by extraction into sodium carbonate solution.

This procedure is more time consuming than the oxidative hydroxylation procedure using lead tetraacetate which is described later. Oxidation of N-oxides substituted with electron-releasing groups (for example,



4-methylquinoline 1-oxide) give low yields of hydroxamic acids. The +I effect of the methyl group would increase the electron density of the ring and the formation of the pseudo-base would be hindered to some extent. These disadvantages limited the use of this method of preparing hydroxamic acids to three compounds that have been previously reported.

An attempt to prepare 1-hydroxy-4-nitro-2(1H)-quinolone by this method gave only a small amount of product which did not give a magenta color with ferric chloride, therefore, was not examined further. Quinoline 1-oxide was converted to 1-hydroxy-2(1H)-quinolone in 50% yield. 4-Methylquinoline 1-oxide was converted into the corresponding hydroxamic acid, 1-hydroxy-4-methyl-2(1H)-quinolone in a 25% yield and a similar yield of 2-hydroxy-1(2H)-isoquinolone was obtained from the oxidation of the appropriate N-oxide.

#### Oxidations with lead tetraacetate

In 1962, oxidative hydroxylations were reported using lead tetraacetate instead of alkaline potassium ferricyanide. Ohta and Ochiai reported the successful oxidation of quinoline 1-oxide and various derivatives in yields of 63-81%. Coutts, Pitkethly and Wibberley used this method in 1965 for the preparation of 3-methyl and 3-ethyl-1-hydroxy-2(1H)-quinolones.

This method of preparing hydroxamic acids involves the addition of lead tetraacetate to a solution of the





N-oxide in benzene. The initial product is the acetate (43) and this is hydrolyzed to the hydroxamic acid (44) with 10% hydrochloric acid solution. Each product gave a characteristic magenta color on addition of alcoholic ferric chloride solution. In this way, the formation of a hydroxamate function was confirmed.

#### Oxidation of 4-nitroquinoline 1-oxide

Ohta and Ochiai (1962) reported that oxidation of 4-nitroquinoline 1-oxide with lead tetraacetate in benzene does not yield 1-hydroxy-4-nitro-2(1H)-quinolone. However, when this reaction was repeated, in this study, with a sample of 4-nitroquinoline 1-oxide a product was isolated which had all the characteristics of a hydroxamic acid. The compound obtained was soluble in sodium carbonate solution, gave the characteristic magenta color with ferric chloride solution and the infrared spectrum was typical of a hydroxamic acid. The mass spectrum exhibited lines for  $\underline{M}^+$  at m/e 206 (base peak) and  $(M-16)^+$  at m/e 190 which is consistent for a hydroxamic acid structure (to be discussed later). From these findings it appeared that 1-hydroxy-4-nitro-2(1H)-quinolone had been obtained. However, Ohta and Ochiai's observation left some doubt as to the purity of the starting material used in this study.

A literature search for the melting points of the nitroquinoline 1-oxides revealed that 5-nitroquinoline 1-oxide has a melting point of 155-157° which is uncom-



fortably close to that of 4-nitroquinoline 1-oxide (153-154°). Furthermore, 5-nitroquinoline 1-oxide can be prepared by the same method as 4-nitroquinoline 1-oxide, only at a lower reaction temperature. The yield of the hydroxamic acid obtained with this procedure was low (28%), so the possibility existed that the sample of 4-nitroquinoline 1-oxide used was contaminated with some of the 5-nitro-derivative and that the hydroxamic acid isolated was, in fact, 1-hydroxy-5-nitro-2(1H)-quinolone. Therefore, it was imperative that the authenticity of the 4-nitroquinoline 1-oxide used in this study was established.

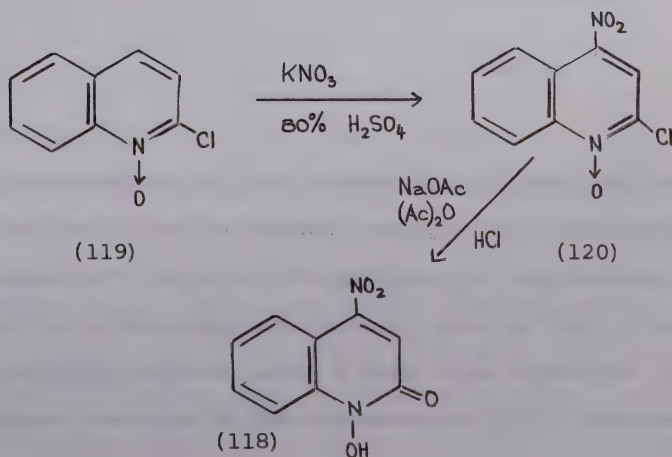
Thin layer chromatography was carried out on the sample of the N-oxide using chloroform as the developing solvent. A compact spot was obtained,  $R_f$  0.78, which was some indication of a pure compound.

4-Nitroquinoline 1-oxide is very reactive to nucleophilic substitution and it is known that the nitro group is readily replaced with a halogen atom. The sample of 4-nitroquinoline 1-oxide used in this study was converted to 4-chloroquinoline 1-oxide in excellent yields when treated with acetyl chloride at 0° or with hot concentrated hydrochloric acid. It is a reasonable assumption that only a 4-nitro group would undergo nucleophilic substitution under these conditions (Okamoto 1951). The sample of 4-chloroquinoline 1-oxide was oxidized with lead tetraacetate and the infrared spectrum of the resul-



ting hydroxamic acid was identical to that of 4-chloro-1-hydroxy-2(1H)-quinolone prepared by a procedure outlined by Yamazaki *et al* (1968). Yamazaki's procedure involved preparing 1-acetoxy-4-chloro-2(1H)-quinolone and hydrolyzing this acetoxy compound with 10% hydrochloric acid solution.

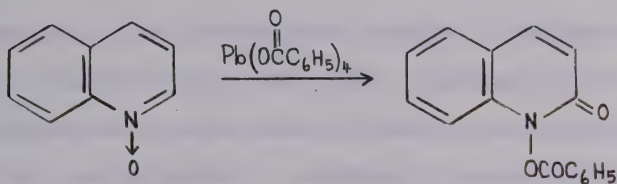
An alternative route for the preparation of 1-hydroxy-4-nitro-2(1H)-quinolone (118) was reported, by Yamazaki *et al* (1968), while the present study was in progress. This procedure was repeated. 2-Chloroquinoline 1-oxide (119) was nitrated with potassium nitrate and 80% sulfuric acid to give 2-chloro-4-nitroquinoline 1-oxide (120). This oxide was first reacted with acetic anhydride and sodium acetate and then with 10% hydrochloric acid, and in this way the hydroxamic acid (118) was obtained in good yield.





The infrared spectra of the hydroxamic acids prepared by these two methods were found to be virtually identical. A sample of 5-nitroquinoline was obtained commercially and oxidized to the N-oxide. Further oxidation with lead tetraacetate yielded 1-hydroxy-5-nitro-2(1H)-quinolone in low yield. The infrared spectrum of this hydroxamic acid was found to differ significantly from the two described immediately above.

Ohta (1963) carried out the oxidation of quinoline 1-oxide with lead tetrabenzoate and isolated 1-benzoyloxy-2(1H)-quinolone (121). Ohta and Ochiai (1962) assumed that the corresponding 1-acetoxy compound (43) must be

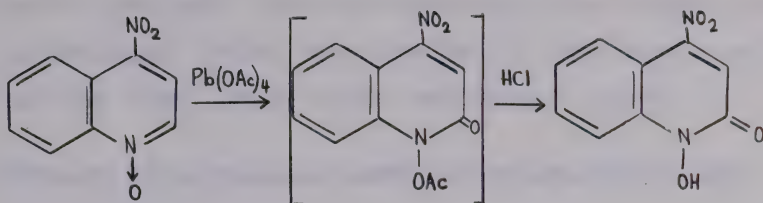


(121)

the intermediate in lead tetraacetate oxidations and that the acylation of the oxygen atom of the N-oxide group was an important factor in the progress of the reaction. An electron withdrawing substituent, such as the nitro group, in the para-position would hinder this acylation. An infrared spectrum of the intermediate (122) obtained on







(122)

oxidation of 4-nitroquinoline 1-oxide, in this study, indicates that it is mainly the N-acetoxy compound. Strong absorption bands are noticed at 1812 and 1685  $\text{cm}^{-1}$  indicative of an N-acetoxy (Loudon and Wellings, 1960) and a carbonyl group respectively.

Oxidative hydroxylation of 3-nitroquinoline 1-oxide with lead tetraacetate yielded 1-hydroxy-3-nitro-2(1H)-quinolone in a 62% yield. Similarly, the following hydroxamic acids were obtained from related N-oxides, in the yields stated: 1-hydroxy-5-nitro-2(1H)-quinolone (23%); 1-hydroxy-6-nitro-2(1H)-quinolone (46%); 1-hydroxy-3-methyl-2(1H)-quinolone (56%); 1-hydroxy-3,6-dimethyl-2(1H)-quinolone (46%); 1-hydroxy-3,7-dimethyl-2(1H)-quinolone (46%); 1-hydroxy-6,7-dimethyl-2(1H)-quinolone (49%); 4-chloro-1-hydroxy-2(1H)-quinolone (27%).

#### Preparation of 1-acetoxy-4-chloro-2(1H)-quinolone

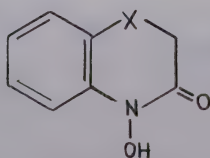
This compound was prepared in good yield by reacting 2-chloro-4-nitroquinoline 1-oxide with acetyl chloride. The infrared spectrum of the acetate had an absorption



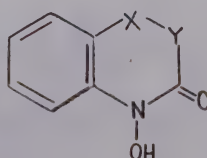
band at  $1800\text{ cm}^{-1}$ . This is indicative of an  $\text{>N-OAc}$  group-  
ing (Paquette, 1965a; Ohta and Ochiai, 1962; Loudon and  
Wellings, 1960, 1960a; Loudon and Tennant, 1960).

Reductive cyclizations by means of sodium borohydride  
and palladium-charcoal

Sodium borohydride in the presence of palladium-  
charcoal is a reducing system which has been used in the  
preparation of many cyclic hydroxamic acids. Coutts and  
Wibberley (1964) were able to show that o-nitro-esters,  
in which the ester group was suitably orientated with  
respect to the o-nitrophenyl group, were reduced in good  
yields to cyclic hydroxamic acids on treatment with this  
reducing system. This method was capable of wide applic-  
ation, and various ring systems have been prepared (Coutts  
and Wibberley, 1963; Coutts, Noble and Wibberley, 1964;  
Coutts, Peel and Smith, 1965; Coutts and Hindmarsh, 1966;  
Coutts and Smith, 1967) including hydroxamic acid deriv-  
atives of quinoline (123;  $\text{X-Y=CH=CH}$ ), oxindole (124),  
benzothiazine (91), benzothiazine 1,1-dioxide (125;  
 $\text{X=SO}_2$ ), benzoxazine (90), quinoxaline (93), quinoxaline  
(92) and triazanaphthalene (126).

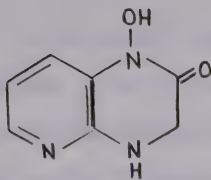


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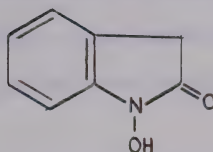


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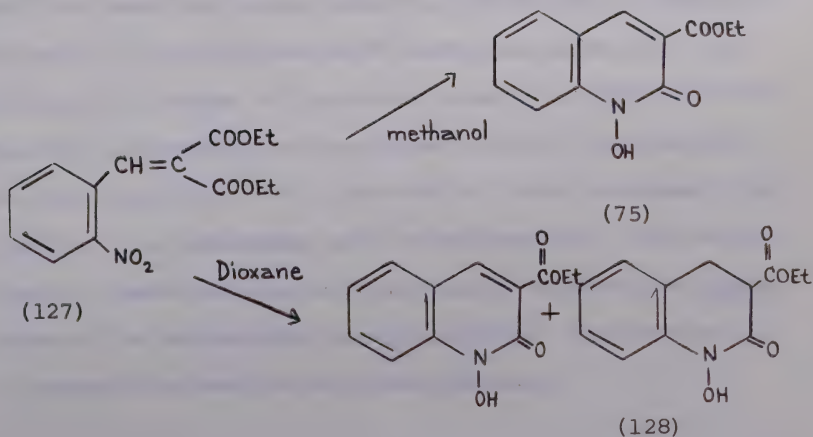


(126)



(124)

Because some hydroxamic acids of these types were needed for NMR and mass spectrometry studies, a few were prepared by reductive cyclization of suitable esters. Reduction of a solution of ethyl o-nitrobenzylidene-malonate (127) in methanol with sodium borohydride and palladium-charcoal gave ethyl 1-hydroxy-2(1H)-quinolone-3-carboxylate (75). In contrast, ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate (128) was the product of the reduction of the same ester in dioxane. This interesting solvent effect has been reported by Coutts (1969).





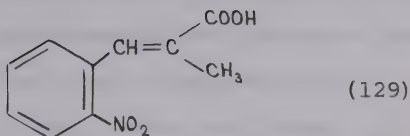
1-Hydroxy-2(1H)-quinolone-3-carboxylic acid (75, Et replaced with H) was readily prepared in a good yield by saponifying ethyl 1-hydroxy-2(1H)-quinolone-3-carboxylate with sodium hydroxide solution.

Coutts, Pitkethly and Wibberley (1965) obtained 1-hydroxy-3-methyl-2-(1H)quinolone (73) by a long procedure involving heating a solution of isatin and propionaldoxime in 40% potassium hydroxide solution. The resulting 3-methylquinoline-4-carboxylic acid was decarboxylated to give 3-methylquinoline which was successively oxidized with hydrogen peroxide, then lead tetraacetate, to give the required hydroxamic acid.

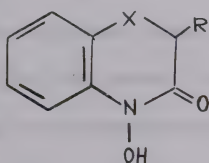
A simpler method was sought.  $\beta$ -o-Nitrophenyl- $\alpha$ -methylacrylic acid (129) was obtained using the conditions described by Cunningham et al (1949), in which o-nitro-benzaldehyde, propionic anhydride and sodium propionate were allowed to react at 150°. The acid was esterified and the resulting ester reduced. Reduction of one gram of ethyl  $\beta$ -o-nitrophenyl- $\alpha$ -methylacrylate gave only fifteen milligrams of 1-hydroxy-3-methyl-2(1H)-quinolone. Neither the raising of the temperature of the reaction nor the application of ultra-violet light increased the yield of the hydroxamic acid significantly. This method does not seem satisfactory presumably because the trans geometry is not amenable to cyclization. This method of preparation was not examined further.







Reduction of methyl  $\alpha$ -(o-nitrophenylthio)propionate gave 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine (130; X=S, R=CH<sub>3</sub>).



(130)

A convenient method for the preparation of a sulfone is to oxidize the appropriate sulfide with potassium permanganate at room temperature (Coutts and Smith, 1967). Using their procedure, methyl  $\alpha$ -(o-nitrophenylthio)propionate was converted to the corresponding sulfone, methyl  $\alpha$ -(o-nitrobenzenesulfonyl)propionate. Methyl  $\alpha$ -(o-nitrobenzenesulfonyl)acetate was prepared similarly.

Two benzothiazine 1,1-dioxide hydroxamic acids were easily obtained by the reductive cyclization method using sodium borohydride and palladium-charcoal. Methyl  $\alpha$ -(o-nitrobenzenesulfonyl)propionate was reduced to 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine



1,1-dioxide (130;  $X=SO_2$ ;  $R=CH_3$ ) and similarly reduction of methyl  $\alpha$ -(o-nitrobenzenesulfonyl)acetate gave 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (130;  $X=SO_2$ ;  $R=H$ ). When the latter was treated with pyridinium bromide perbromide the 2-bromo compound was obtained in an excellent yield (130;  $X=SO_2$ ;  $R=Br$ ). The NMR spectrum showed a 1-proton singlet at  $\tau$  3.01 ( $-CH_2-Br$ ) which was exchanged when  $D_2O$  was added to the sample. The N-OH proton was included in the aromatic region. This bromo compound does have some antibacterial properties (to be discussed later) which can be contrasted with the non-brominated compound and the related methyl compound.

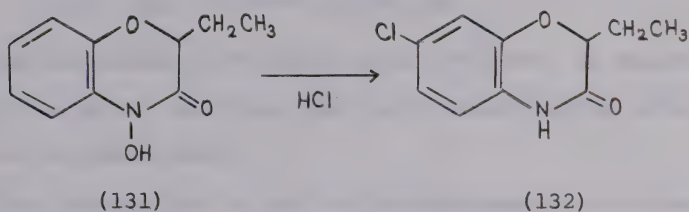
#### Preparation of benzoxazine hydroxamic acids

The preparation of 2H-1,4-benzoxazine hydroxamic acids by the reductive cyclization of  $\alpha$ -o-nitrophenoxy esters has been reported by Coutts and Hindmarsh (1966). Two of the hydroxamic acids necessary for the present study were prepared by this reported method.

Ethyl  $\alpha$ -(o-nitrophenoxy)hexanoate and ethyl  $\alpha$ -(o-nitrophenoxy)butyrate were synthesized from the sodium salt of o-nitrophenol and the appropriate 2-bromo-ester. Reduction of these esters yielded 2-n-butyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (130;  $X=O$ ,  $R=\underline{n}$ -butyl) and 2-ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (131) respectively.



Preparation of 7-chloro-2-ethyl-3,4-dihydro-3-oxo-2H-1,4-benzoxazine

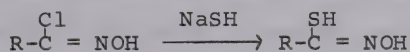


Purification of a small sample of 2-ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (131) was attempted by first dissolving the hydroxamic acid in sodium carbonate solution then acidifying the filtrate with hydrochloric acid. Unexpectedly, this procedure gave a precipitate which did not give the magenta color with ferric chloride characteristic of hydroxamic acids. A mass spectrum of the product indicated that the structure contained chlorine and was assumed to be a chloro-lactam (132) as the infrared spectrum had absorption peaks at  $3190\text{ cm}^{-1}$  (NH) and  $1696\text{ cm}^{-1}$  (C=O). The position of the chlorine has been identified as being in the 7-position (Coutts and Pound, 1969). This reaction of hydroxamic acids with hydrochloric acid is not restricted to the benzoxazines. Coutts and Pound have found similar chloro-lactam formations with the 2H-1,4-benzothiazines.



### Attempted Preparations of Thiohydroxamic Acids

The synthesis of acyclic thiohydroxamic acids has been achieved by the interaction of sodium hydrosulfide and chlorohydroxamic acids (Bacchetti and Alemagna, 1958),



and from the esters of thionic acids ( $\text{R}-\overset{\text{S}}{\underset{\text{=}}{\text{C}}}-\text{OEt}$ ) or esters of dithioacids by reacting them with hydroxylamine (Mizukami and Nagata, 1966).

The preparation of cyclic thiohydroxamic acids was first reported by Shaw et al in 1950. 1-Hydroxy-2(1H)-pyridone (15) possessed antibacterial properties and Shaw and co-workers wished to compare the activity of this pyridone with that of the sulfur analog. Treatment of 2-bromopyridine with sodium sulfide or sodium hydrosulfide resulted in the formation of the thiohydroxamic acid, since the bromine atom in 2-bromopyridine 1-oxide is considerably more reactive than 2-bromopyridine itself. In addition several ring substituted derivatives were similarly prepared. The synthesis of 2-mercaptopyridine 1-oxide was also accomplished when 2-bromopyridine 1-oxide was reacted with thiourea. A similar preparation was reported by Semenov and Dolliner (1957). 2-Chloropyridine 1-oxide when treated with sodium sulfide and sodium hydrosulfide also is converted to the same thiohydroxamic acid (McLure and Sherman, 1965). These cyclic thiohydroxamic acids had a far more potent antibacterial activity in vitro against some organisms than the cor-

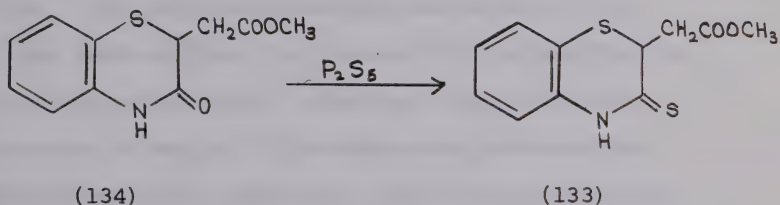




responding 1-hydroxy-2(1H)-pyridone derivatives.

The syntheses of various substituted quinoline thiohydroxamic acids have not been reported and because the pyridine thiohydroxamic acids were found to be so much more potent against bacteria, attempts were made at synthesizing various thiohydroxamic acids.

Bourdais (1965), in his work on lactams, reported the synthesis of methyl (3,4-dihydro-3-thioxo-2H-1,4-benzothiazin-2-yl)acetate (133) from methyl (3,4-dihydro-3-oxo-2H-1,4-benzothiazin-2-yl)acetate (134) by treating the latter with phosphorous pentasulfide in toluene. The same procedure was applied in the present study.

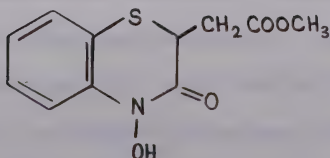


1-Hydroxy-2(1H)-quinolone, 4-hydroxy-1(2H)-iso-quinolone and 1-hydroxy-4-methyl-2(1H)-quinolone were reacted with phosphorous pentasulfide according to Bourdais' procedure. Solid products were obtained which did not give any color change on the addition of ferric chloride solution. This observation was compared with the result of treating an authentic sample of 2-mercaptopyridine 1-oxide with ferric chloride which gave a dark blue color. From this



observation it was apparent that quinoline thiohydroxamic acids had not been obtained. Infrared spectra showed that C=S stretching and N-H stretching bands were present in the products of the phosphorous pentasulfide reactions. This suggested that thiolactams had probably been obtained. A literature survey revealed the following melting points for the lactams: 2-mercaptoquinoline,  $174^{\circ}$ ; 1-mercaptoisoquinoline,  $171^{\circ}$ ; 2-mercapto-4-methylquinoline,  $253^{\circ}$ . The products obtained in this study melted at  $170-173^{\circ}$ ,  $167-168^{\circ}$  and  $258-260^{\circ}$  respectively. Confirmation that the thiolactams were obtained was made by treating 2(1H)-quinolone with phosphorous pentasulfide. The infrared spectrum was identical to the spectrum obtained from the product of the reaction of phosphorous pentasulfide and 1-hydroxy-2(1H)-quinolone.

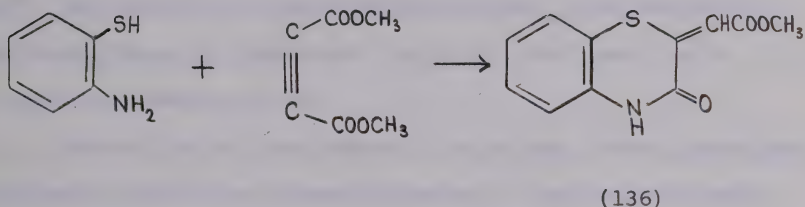
Treatment of methyl (3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazin-2-yl)acetate (135) with phosphorous pentasulfide gave an oily product which gave a magenta color with ferric chloride solution. A solid product was



(135)



precipitated on trituration of the oil with ethanol. This product melted at 257-258°, did not give any color change on the addition of ferric chloride solution and gave a molecular ion at m/e 235 in the mass spectrum. The mass spectral evidence indicated the possibility that methyl (3,4-dihydro-3-oxo-2H-1,4-benzothiazine)  $\Delta^{2,\alpha}$  acetate (136) was formed. This compound was prepared by an alternative method (Kalbag et al, 1967) in which ether solutions of 2-aminothiophenol and dimethylacetylenedicarboxylate were mixed. The product obtained from this lat-



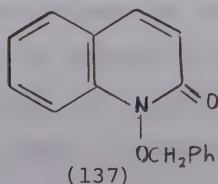
ter reaction gave an infrared spectrum virtually identical with that of the compound isolated from the phosphorous pentasulfide reaction. The oil remaining after the removal of (136) was taken up into ether and extracted with sodium carbonate solution. Acidification of the aqueous layer gave starting material. Evaporation of the ether produced a solid, m.p. 138-140°, which was not a hydroxamic acid; no purple color was observed on the addition of ferric chloride solution. Its infrared spectrum showed absorption peaks at 3200, 1745, and 1670  $\text{cm}^{-1}$



which are ascribable to N-H, ester carbonyl and lactam carbonyl groups respectively. The compound, therefore, was deduced to be methyl (3,4-dihydro-3-oxo-2H-1,4-benzothiazin-2-yl)acetate (134). Bourdais (1962) reported the melting point for this compound (134) to be 145-146°.

Three other hydroxamic acids were subjected to treatment with phosphorous pentasulfide; they were 1,4-dihydroxy-2(1H)-quinolone, 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzoxazine and 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzothiazine. Treatment of the first of these compounds yielded a solid product which melted over 300°, but which could not be identified. The benzoxazine and benzothiazine hydroxamic acids did not react with phosphorous pentasulfide.

Protection of the hydroxyl group was investigated and the benzyl group was chosen because it can be relatively easily removed by catalytic hydrogenation. Attempts to synthesize 1-benzyloxy-2(1H)-quinolone (137) by boiling under reflux 1-hydroxy-2(1H)-quinolone and benzyl chloride, firstly, in water, and secondly, in ethanolic sodium hydroxide, resulted in recovery of unchanged starting materials. The reaction of the







sodium salt of 1-hydroxy-2(1H)-quinolone and benzyl chloride in acetone was a successful method for preparing the required product. Treatment of 1-benzyloxy-2(1H)-quinolone with phosphorous pentasulfide resulted in the removal of the protective group and in the isolation of 2-mercaptoquinoline regardless of the reaction time and solvent used. Klingsberg and Papa (1951) reported that toluene and benzene have limited value in the thiation of pyridones and suggest the use of pyridine. The reaction was repeated in this study using pyridine as the solvent. Unchanged starting material was recovered after a reflux period of four and one half hours. Increasing the reflux time to fifty five hours resulted in the formation of 2-mercaptoquinoline.

Because thiohydroxamic acids can not be prepared directly from hydroxamic acids by reaction with phosphorous pentasulfide a different approach was required. The synthesis of 4-mercaptoquinoline 1-oxide has been reported in the literature by Itai (1949) and Suzuki (1961). Suzuki obtained the desired compound by reacting 4-bromoquinoline 1-oxide with sodium sulfide and Itai obtained the same product by reacting 4-chloroquinoline 1-oxide with thiourea and sodium hydroxide.

Thus, thiourea was allowed to react with 2-chloroquinoline 1-oxide, in this present study, and 2-mercaptoquinoline was the only product isolated. However, when sodium sulfhydrate was added to 2-chloroquinoline 1-oxide a solid product was obtained which gave a dark blue color



with ferric chloride solution. This is reminiscent of the color obtained on the addition of ferric chloride solution to 2-mercaptopyridine 1-oxide. The infrared spectrum of the product showed a strong absorption band at  $1180\text{ cm}^{-1}$  which could be due to C=S stretching. These observations suggested that 2-mercaptoquinoline 1-oxide had been obtained. The use of derivatives of 2-mercaptopyridine 1-oxide and 2-mercaptoquinoline 1-oxide for the treatment of superficial mycoses and as active antiinfection agents has been patented (Conover, English and Larrabee, 1960) but 2-mercaptoquinoline 1-oxide itself was not described.



## EXPERIMENTAL



## EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus. All the melting points and boiling points quoted are uncorrected. Infrared spectra were recorded on either a Beckman IR10 Spectrophotometer or a Beckman IR5A Spectrophotometer; nuclear magnetic resonance (NMR) spectra were taken on a Varian A-60 Spectrophotometer. Mass spectra were determined by Dr. A.M. Hogg and his associates with an AEI MS-9 mass spectrometer at an ionizing potential of 70 eV. The direct probe method was used. Elemental analyses were done by the Department of Chemistry and Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta. The dimethylquinoline samples used in this study were provided by Dr. W.A. Ayer, Department of Chemistry, University of Alberta.

Preparation of Alkylquinolines

$\beta$ -o-Nitrophenyl- $\alpha$ -methylhydracrylaldehyde (Willimott Simpson, 1926)

Method A

o-Nitrobenzaldehyde (15.1 g) was dissolved in ethanol (20 ml). To this solution was added propionaldehyde (8.7 g) and piperidine (a few drops). The mixture was allowed to stand for thirteen days and then flooded with water and extracted with ether. The ether portion was dried over sod-





ium carbonate and evaporated to yield a viscous yellow oil (17.2 g). Mass spectrum,  $m/e$ : 162 (2.0%), 152 (4.6%).

IR spectrum (thin film): 3700-2200 (OH); 1680 (C=O); 1335, 1515  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Thin layer chromatography of the oil: the oil was taken up into acetone and spotted on silica gel plates (Silica Gel G, Merck). Two components were obtained using the developing solvent system benzene:methanol (10:1.5),  $R_f$  0.77 and 0.63. The major component was the latter one.

The 2,4-dinitrophenylhydrazine derivative of the product had m.p. 212-214°. The IR spectrum (KBr disc) of this derivative had absorption peaks at 3650-3300 (OH), 3250 (NH), 1616 (C=N), 1515 and 1330  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Anal. Found: C, 48.80; H, 4.45.  $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_7$  requires: C, 49.36; H, 3.85.

#### Method B

o-Nitrobenzaldehyde (10 g) and propionaldehyde (5.7 g) were added to a 3% aqueous solution of barium hydroxide (180 ml) and the mixture was left to stand at room temperature for twenty two hours. Additional water (100 ml) was added and the whole extracted with ether. On evaporation of the ether a reddish-brown oil was obtained (7 g).

IR spectrum (thin film): 3700-2500 (OH); 1680 (C=O); 1340, 1525  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Thin layer chromatography of the product: the oil



was spotted on silica gel plates (Silica Gel G, Merck) and four components ( $R_f$  0.69, 0.40, 0.28 and 0.10) were obtained using benzene:chloroform (100:1) as the developing solvent. The leading component ( $R_f$  0.69) was removed from the plate and taken up into ether. Evaporation of the ether yielded a solid which was identified by IR spectrum as o-nitrobenzaldehyde. Because of the impurity of this oil it was not further investigated.

Treatment of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde with stannous chloride and concentrated hydrochloric acid

To crude  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde (1 g) (prepared by Method A, page 89) was added concentrated hydrochloric acid (25 ml) and stannous chloride (10 g). The mixture was boiled under reflux for one hour and then cooled. The cooled dark red solution was made alkaline and extracted with ether. The ether solution was shaken with dilute hydrochloric acid and the base after being liberated was again dissolved in ether. Evaporation of the ether yielded a basic oil (0.12 g) which was identified as 3-methylquinoline by the IR spectrum, but because of the small yield it was not purified.

Attempted oxidation of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde with potassium permanganate

To crude  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde (1 g) in glacial acetic acid (20 ml) was added a solution of potassium permanganate (2 g) in water (5 ml)



and the whole was stirred, at room temperature, for sixteen and one-half hours. Sufficient 30% hydrogen peroxide was added dropwise until the solution became clear. The solution was neutralized with sodium carbonate, flooded with water and extracted with ether. Evaporation of the ether yielded a yellow oil (0.95 g) which had an IR spectrum identical to that of the starting material.

Attempted oxidation of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde with silver nitrate solution

To a solution of crude  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde (1 g) in ethanol (200 ml) was added silver nitrate (1.8 g) in water (10 ml). Sodium hydroxide solution (0.5 N, 44 ml) was added dropwise with stirring and the reaction mixture was allowed to stir, at room temperature, overnight. The insoluble material was removed by filtration and the solution was concentrated to a small volume under vacuum. The alkaline solution was extracted with ether and then acidified with 10% hydrochloric acid. A white precipitate formed and was collected by filtration (70 mg). The IR spectrum of this product was identical with that of o-nitrobenzoic acid. The non-acidic material (0.4 g) was identified as starting material by the IR spectrum.

Reduction of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde with sodium borohydride and palladium-charcoal

Sodium borohydride (1.5 g) was dissolved in water (5 ml) and palladium-charcoal (0.1 g) suspended in water





(5 ml) was carefully added. The suspension was diluted with dioxane (10 ml) and cooled using an ice-bath. Nitrogen was bubbled through the mixture while crude  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde (2 g) was added over a thirty minute period and the passage of nitrogen was continued for thirty minutes after the addition had been completed. The mixture was filtered, acidified, diluted with water and extracted with ether. Removal of the ether layer yielded a reddish oil (0.84 g). The IR spectrum revealed the absence of nitro absorption. The oil was taken up into ether and extracted with 5% hydrochloric acid and then 10% sodium hydroxide solution. Evaporation of the ether layer yielded a neutral oil (0.1 g). Basifying the hydrochloric acid portion, extraction with ether and evaporation, yielded the basic oily material (0.33 g). Acidification of the sodium hydroxide portion, extraction with ether and evaporation, yielded the acidic oily material (0.01 g). Thin layer chromatography of these products on silica gel G plates using benzene:methanol (10:1.5) as the developing solvent revealed the presence of twenty-three components:

Neutral material: nine components with  $R_f$  values, 0.00, 0.13, 0.22, 0.27, 0.32, 0.36, 0.48, 0.84, 0.93.

Basic material: six components with  $R_f$  values, 0.15, 0.26, 0.37, 0.48, 0.67, 0.86.

Acidic material: eight components with  $R_f$  values, 0.02, 0.06, 0.12, 0.19, 0.29, 0.32, 0.35, 0.37.





Gas Chromatography of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde

Method A

A sample (8  $\mu$ l) of crude  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde was gas-chromatographed (F and M Model 500 Gas Chromatograph) and the retention times of the two major components present were obtained when

- a) a Carbowax column 20M on Diatomaceous earth was used (6 ft x 1/4 in)
- b) the column temperature was 175°
- c) the injection port temperature was 230°
- d) the helium flow rate was 60 ml/minute

Component one had a retention time of 0.75 minutes.

Component two had a retention time of 2.12 minutes.

When the column temperature was reduced to 125° the retention times were 1.25 and 4.87 minutes respectively.

The components were collected using an open-ended melting-point capillary tube inserted through a hole in a rubber septum installed at exit tip of the chromatograph. The IR spectra of the two components were almost identical, having absorption peaks at 1690 (C=O) and 1345, 1525  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Mass spectrum: the heaviest abundant ion (42%) was at  $m/e$  263. Ions of less than 3% relative abundance were found at  $m/e$  281 and 336. A meaningful interpretation of this spectrum could not be made.



### Method B

A sample (0.02 ml) in ether of  $\beta$ -o-nitrophenyl- $\alpha$ -methylhydracrylaldehyde was gas-chromatographed (F and M Model 500 Gas Chromatograph) and the retention times of the three major components present were obtained when

- a) an SE 30 (10%) on Diaport S column was used (6 ft x 1/4 in)
- b) the injection port temperature was 255°
- c) the helium flow rate was 60 ml/minute
- d) the column temperature was 150°, 200° and 225°

Component one had retention times of 1.14, 0.94 and 0.63 minutes respectively.

Component two had retention times of 2.87, 2.50 and 1.60 minutes respectively.

Component three had retention times of 11.00, 8.00 and 3.00 minutes respectively.

Components two and three were the major components and were collected using an open-ended melting-point capillary tube inserted through a hole in a rubber septum installed at the exit tip of the chromatograph. Component two was identified by IR spectrum to be o-nitrobenzaldehyde. The IR spectrum of component three showed absorption peaks at 1685 (C=O) and 1338, 1520  $\text{cm}^{-1}$  ( $\text{NO}_2$ ). Mass spectrum:  $m/e$  162. No definite conclusions as to the identity of this component could be made.

Components one and two were identified as propionaldehyde and o-nitrobenzaldehyde by injection of authentic



samples (in ether) of these two substances. Peak augmentation occurred.

3-Methylquinoline-4-carboxylic acid (Ornstein, 1907)

To a solution of isatin (15 g) in 40% potassium hydroxide (120 g) was added propionaldoxime (4.6 g) and the mixture was heated ( $100^{\circ}$ ) for nineteen hours. On cooling the reaction mixture a few crystals precipitated out and the yield was increased by concentrating the solution under vacuum. The crystals were collected by filtration and dissolved in water. Acidification of the aqueous layer and filtration yielded the title compound (4 g), m.p.  $246^{\circ}$ . Reported (Coutts, Pitkethly and Wibberley, 1965) m.p.  $250-252^{\circ}$ ; (Ornstein, 1907) m.p.  $254^{\circ}$ .

IR spectrum (Nujol mull):  $3650-2000$  (OH);  $1730\text{ cm}^{-1}$  (C=O).

Propionaldoxime (Beech, 1954)

Hydroxylamine hydrochloride (35 g) in water (34 ml) was treated with sodium carbonate (28 g) in water (100 ml) and the solution kept at  $5-10^{\circ}$  while propionaldehyde (29 g) was added gradually. After two hours the oxime was extracted with ether. The dried ether solution was evaporated to yield the title compound (29.4 g), b.p.  $130-132^{\circ}$ . Reported (Beech, 1954) b.p.  $130-132^{\circ}$ .

IR spectrum (thin film):  $3500-2400$  (OH);  $1635\text{ cm}^{-1}$  (C=N).



### 3-Methylquinoline

#### Method A (Coutts, et al, 1965)

A suspension of 3-methylquinoline-4-carboxylic acid (5 g) and copper powder (1 g) in diphenyl ether (100 g) was boiled under reflux for two hours. The mixture was diluted with dry ether, filtered, and diluted to approximately 500 ml with more ether. Dry hydrogen chloride was passed into the ether solution to precipitate the 3-alkyquinoline hydrochloride. Upon filtering the mixture and dissolving the residue in water, the quinoline was obtained by making the solution alkaline with sodium hydroxide and extracting with ether. Evaporation of the ether yielded an oil (0.1 g) but because of the low yield it was not investigated further.

#### Method B (Manske, 1942)

Ferrous sulphate (15 g), aniline (33 g), o-nitrophenol (20 g) and sulfuric acid (44 g) were maintained at 140-150° and methacrylaldehyde (32 g) was added over a ten minute period. The mixture was heated for an additional three and one-half hours and after this time, the mixture was diluted with water and steam distilled to remove the excess o-nitrophenol. The mixture was then basified with sodium hydroxide solution and steam distillation of the basic material resulted in collection of a yellow oil (17.2 g). Vacuum distillation of this oil yielded aniline (11.2 g) at 40-43° (1 mm) and the title compound (5.8 g)







at 88-90° (1 mm). Reported (Coutts et al, 1965) b.p. 100°/1.5 mm.

### 3-Ethylquinoline-4-carboxylic acid (Mulert, 1906)

A procedure similar to that described for 3-methylquinoline-4-carboxylic acid was used. Isatin (10 g), butyraldoxime (3.7 g) and 50% potassium hydroxide solution (80 g) were heated at 100° for sixty eight hours. Concentration (under vacuum) resulted in the precipitation of crystals which were dissolved in water and acidification yielded the title compound (1.4 g), m.p. 218°. Reported (Coutts et al, 1965) m.p. 218-220°; (Mulert, 1906) m.p. 222°.

IR spectrum (Nujol mull): 3600-2000 (OH); 1725 cm<sup>-1</sup> (C=O).

### Ethyl ethylmalonanilate

Aniline (18 g) was boiled with diethyl ethylmalonate for four hours and ethanol was collected during this period by distillation. The mixture was cooled and ethyl malonanilide (m.p. 206-210°) was collected by filtration. The filtrate was added to excess water and set aside in the refrigerator to crystallize. The crystals (10.6 g) were collected by filtration, taken up into benzene and passed through a silica gel column (1 3/4 cm x 10 cm). Evaporation of the benzene eluate yielded the title compound, m.p. 56-57°.

Anal. Found: C, 66.41; H, 7.47; N, 5.60. Calcd. for



$C_{13}H_{17}NO_3$ : C, 66.38; H, 7.23; N, 5.95.

IR spectrum (Nujol mull): 3240 (NH); 1772 (ester C=O); 1665  $cm^{-1}$  (amide C=O).

NMR spectrum ( $CCl_4$ ): 1-proton singlet at  $\tau$  1.14 for NH; a 1-proton triplet centered at  $\tau$  6.78 for  $-CH-CH_2-$   
 $CH_3$ ; a 2-proton quintet centered at  $\tau$  8.05 for  $-CH-CH_2-CH_3$ ; a  
 3-proton triplet centered at  $\tau$  9.05 for  $-CH-CH_2-CH_3$ ; a  
 3-proton triplet centered at  $\tau$  8.75 for  $-COOCH_2CH_3$ ; a 2-  
 proton quartet centered at  $\tau$  5.85 for  $-COOCH_2CH_3$ .

#### Reaction of thionyl chloride with ethyl ethylmalonanilate

Ethyl ethylmalonanilate (2.3 g) and thionyl chloride (5 ml) were boiled under reflux for twenty one hours in chloroform (20 ml). The chloroform and most of the thionyl chloride were removed under vacuum and water was added to the remaining oil. Extraction of the aqueous layer with ether and evaporation yielded an oil (1 g). The IR spectrum of the oil showed an absorption peak at 1730  $cm^{-1}$  (ester C=O). The oil was taken up into ether and extracted with 10% sodium hydroxide solution and 10% hydrochloric acid solution. No basic material was obtained from the hydrochloric acid extract and the product was not further investigated.

#### Reaction of phosphorous oxychloride with ethyl ethylmalonanilate

##### Method A (Draper and MacLean, 1968)

Ethyl ethylmalonanilate (10 g) was boiled under re-



flux with phosphorous oxychloride (50 g) for ten hours. The mixture was cooled, poured onto crushed ice, basified and extracted with ether. Removal of the ether yielded an oil (5.14 g). The IR spectrum showed absorption peaks at 1812 ( $\overset{\text{O}}{\text{C}}\text{-Cl}$ ), 1730 (ester  $\text{C=O}$ ) and  $1640\text{ cm}^{-1}$  (amide  $\text{C=O}$ ). It appeared that cyclization had not occurred so the oil was not investigated further.

#### Method B

Ethyl ethylmalonanilate (7.5 g) and phosphorous oxychloride (40 g) were boiled under reflux for sixty one hours and worked up as in Method A to yield an oil (3.5 g). The IR spectrum was identical with that obtained in Method A. The oil was taken up into ether and extracted with 5% hydrochloric acid solution and on basifying this extract, extracting with ether and evaporation, an oil was obtained (0.05 g). The IR spectrum showed ester absorption ( $1745\text{ cm}^{-1}$ ) and the oil was not further examined.

#### Reaction of phosphorous pentachloride with ethyl ethylmalonanilate

Ethyl ethylmalonanilate (5 g) was boiled under reflux with phosphorous pentachloride (20 g) for twenty one hours. The reaction mixture was cooled, added to water and extracted with ether. The ether layer was extracted with 5% hydrochloric acid. Basifying the aqueous layer, extraction with ether and evaporation of the ether yielded



an oil (0.01 g). Ester absorption was noticed in the IR spectrum ( $1750\text{ cm}^{-1}$ ). This product was not further examined.

### Ethyl N-phenacetylanthranilate

#### Method A

Ethyl anthranilate (10 g) was added to 10% sodium hydroxide solution (200 ml) and phenyl acetyl chloride (20 g) was added slowly with stirring. The mixture was stirred for a further twenty minutes and the title compound was collected by filtration (15.6 g). The product had m.p.  $58.5-60^{\circ}$ . Reported (deDiesbach et al, 1951) m.p.  $62^{\circ}$ .

Anal. Found: C, 72.33; H, 6.25. Calcd. for  $\text{C}_{17}\text{H}_{17}\text{NO}_3$ : C, 72.08; H, 6.01.

#### Method B (deDiesbach et al, 1951)

Phenyl acetyl chloride (15 g) was added slowly, with stirring, to a solution of ethyl anthranilate (15 g) in ether (100 ml). An immediate precipitate formed which was collected by filtration (9.2 g). The product had m.p.  $160-162^{\circ}$ . The IR spectrum had a band between  $2950$  and  $2500\text{ cm}^{-1}$  and ester carbonyl absorption at  $1700\text{ cm}^{-1}$ . These observations suggested that the product was the hydrochloric acid salt of ethyl anthranilate. Reported (Heilbron et al, 1965) m.p.  $170^{\circ}$ .

### 2,4-Dihydroxy-3-phenylquinoline

To a solution of ethyl N-phenacetylanthranilate







(16 g) in dry toluene (200 ml) was added sodium metal (6 g) in toluene (100 ml) and the mixture was boiled under reflux for four hours. The reaction mixture was cooled, filtered and the residue immediately dissolved in dry ethanol to decompose any unreacted sodium metal. Evaporation of the ethanol yielded the title compound (10.1 g), m.p. 330-332° (acetic acid). Reported (Diesbach et al, 1951) m.p. 318°.

Anal. Found: C, 75.76; H, 4.95. Calcd. for  $C_{15}H_{11}NO_2$ : C, 75.94; H, 4.64.

#### 2,4-Dichloro-3-phenylquinoline

Phosphorous oxychloride (40 ml) was added to 2,4-dihydroxy-3-phenylquinoline (6 g) and the mixture was boiled under reflux, with stirring, for fifteen hours. The reaction mixture was cooled, added to ice water and stirred for four hours. The product was collected by filtration and was obtained in a 99.8% yield, m.p. 85-87°. Reported (German Patent, 1930) m.p. 94°.

$M^+$  (mass spectrum): 273.

#### Treatment of 2,4-dichloro-3-phenylquinoline with tin and hydrochloric acid

Tin metal (20 g) was added to a solution of 2,4-dichloro-3-phenylquinoline (2.5 g) in concentrated hydrochloric acid (55 ml) and the mixture was boiled under reflux for one hour. The mixture was cooled, filtered and made basic with 50% sodium hydroxide. A precipitate was



collected by filtration which was boiled under reflux in 50% sodium hydroxide for one-half hour. The reaction mixture was cooled, filtered, the filtrate was diluted with water and extracted with ether. A yellow solid was obtained on evaporation of the ether (0.65 g), m.p. 86-88°. The IR spectrum showed an absorption peak at 3390  $\text{cm}^{-1}$  (NH) and the mass spectrum revealed an  $\text{M}^+$  peak at 209. This product was 3-phenyl-1,2,3,4-tetrahydroquinoline. Reported (Archer et al, 1964) m.p. 86-87°; (Hubner, 1908) m.p. 83°.

#### Preparation of N-oxides

##### Quinoline 1-oxide

To quinoline (60 g) was added glacial acetic acid (150 ml) and 30% hydrogen peroxide (50 ml). The mixture was heated at 60-70° for three hours and a further 30 ml of 30% hydrogen peroxide was added. The heating was continued for fifteen hours. The volatile reactants were removed, water (50 ml) added and more volatile material removed. Sufficient potassium carbonate and water were added to form a thick paste and this was allowed to stand overnight. The reaction was filtered and the filtrate extracted with chloroform. The residue obtained on removal of the chloroform was poured into a large quantity of ethylacetate and left to stand overnight to crystallize. The title compound was obtained in 59.3% yield, m.p. 48-52°. Reported (Ochiai, 1953) m.p. 60-62°.



Isoquinoline 2-oxide (Robison and Robison, 1956)

A similar procedure to that for the preparation of quinoline 1-oxide was used to obtain the title compound in 38.1% yield, m.p. 100-104°. Reported (Robison and Robison, 1956) m.p. 105-106°.

4-Methylquinoline 1-oxide

The title compound was obtained in 82.7% yield using the procedure described for the preparation of quinoline 1-oxide. The product had m.p. 119-120.5° (water). Reported (Ochiai et al, 1944) m.p. 121°; (Ochiai and Tanida, 1957) m.p. 113-115°.

4-Nitroquinoline 1-oxide (Horiuchi, 1967)

Method A

Quinoline 1-oxide (10 g) and potassium nitrate (7 g) were added in small portions to concentrated sulfuric acid (6 g) while cooling the flask with water. When the additions were complete the reaction mixture was warmed to between 65-70° for one and one-half hours and then poured into a large amount of ice water with stirring. The product was collected by filtration, washed with water until the washings were no longer acidic, and then with dilute sodium carbonate solution until the washing was almost free from color. The product was again washed with water, dried in air and recrystallized from acetone to give the title compound in 42.8% yield (5.6 g), m.p. 153.5-155°. Reported (Horiuchi, 1967) m.p. 153-154°; (Ochiai, 1953)



m.p.  $159^{\circ}$ . Thin layer chromatography on alumina using chloroform as the developing solvent revealed one component,  $R_f$  0.78.

Anal. Found: C, 56.65; H, 3.54. Calcd. for  $C_9H_6N_2O_3$ : C, 56.84; H, 3.15.

IR spectrum (Nujol mull):  $1335, 1510\text{ cm}^{-1}$  ( $NO_2$ ).

#### Method B (Hamana and Nagayoshi, 1966)

Quinoline 1-oxide (8 g) was added slowly, with stirring, to 80% sulfuric acid (40 ml). Potassium nitrate (6 g) was added and the reaction mixture was allowed to stand at room temperature for five days. The mixture was poured into ice water, with stirring, and the product filtered, washed with water and dilute sodium carbonate solution. The title compound (5.6 g), had m.p.  $149-151^{\circ}$ .

#### 3-Nitroquinoline 1-oxide (Ochiai and Kaneko, 1959)

Benzoyl chloride (9.4 g) was added to an ice-cold solution of quinoline 1-oxide (10 g) dissolved in chloroform (200 ml). The mixture was chilled to  $-20$  to  $-15^{\circ}$  and finely pulverized silver nitrate (13.7 g) was added in small portions, under agitation, over a period of thirty minutes. The reaction mixture was stirred with cooling for five hours and for a further five hours at room temperature. The mixture was allowed to stand for two days, silver chloride was removed by filtration and washed with chloroform. The combined filtrate and washings were washed with a saturated solution of sodium bicarbonate and







then 5% hydrochloric acid. The chloroform solution was dried over anhydrous sodium sulfate and the solvent evaporated. Recrystallization of the residue from acetone gave the title compound (1.7 g, 13.0%), m.p. 189-191°. Reported (Ochiai and Kaneko, 1959) m.p. 189-191°.

Anal. Found: C, 57.29; H, 3.64; N, 15.04. Calcd. for  $C_9H_6N_2O_3$ : C, 56.84; H, 3.15; N, 14.74.

IR spectrum (Nujol mull): 1360, 1525  $cm^{-1}$  ( $NO_2$ ).

#### General procedure for the preparation of N-oxides

A similar procedure to that described by Kaslow and Buchner (1958) was used in which 30% hydrogen peroxide (4 ml) is added to a solution of the appropriate quinoline (0.02 moles) in glacial acetic acid (20 ml). The reaction mixture is warmed between 65-70° for three hours. The mixture is concentrated, basified with saturated sodium carbonate solution (70 ml) and extracted with chloroform. Removal of the chloroform yields the N-oxide which is recrystallized from a suitable solvent.

#### 5-Nitroquinoline 1-oxide

The title compound was obtained in 76.0% yield using the general procedure for the preparation of N-oxides. The product had m.p. 159-161.5° (acetone). Reported (Kataoka et al, 1966) m.p. 161°; (Tanida, 1959) m.p. 160-161°.

Anal. Found: C, 56.69; H, 3.28. Calcd. for  $C_9H_6N_2O_3$ : C, 56.84; H, 3.15.



IR spectrum (Nujol mull): 1335, 1515  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

### 6-Nitroquinoline 1-oxide

The general procedure for the preparation of N-oxides was used and gave a 49.1% yield of the title compound. 6-Nitroquinoline 1-oxide had m.p. 219-221° (acetone). Reported (Kataoka et al, 1966) m.p. 221°.

Anal. Found: C, 57.03; H, 3.11. Calcd. for  $\text{C}_9\text{H}_6\text{N}_2\text{O}_3$ : C, 56.84; H, 3.15.

IR spectrum (Nujol mull): 1350, 1530  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

### Attempted preparation of 8-nitroquinoline 1-oxide

The general procedure for the preparation of N-oxides was used. A solid was obtained (1.15 g) which was recrystallized from acetone giving an immediate red product which was collected by filtration (90 mg). The product had a m.p. 256-257°. The IR spectrum (Nujol mull) had the following absorption bands: 3200 (NH), 1730 (C=O), 1350, 1515, 845  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).  $\underline{\text{M}}^+$  (mass spectrum): 178 and an accurate mass determination revealed the elemental composition  $\text{C}_8\text{H}_6\text{N}_2\text{O}_3$ . The product was assumed to be 7-nitrooxindole.

Anal. Found: C, 53.95; H, 3.16; N, 15.46.  $\text{C}_8\text{H}_6\text{N}_2\text{O}_3$  requires: C, 53.93; H, 3.37; N, 15.73.

Evaporation of the acetone yielded recovery of the starting material, 8-nitroquinoline.

### 3-Methylquinoline 1-oxide

The general procedure for the preparation of N-



oxides was used. A 34.4% yield of the title compound was obtained. The product had m.p. 81-83°. Reported (Coutts *et al*) m.p. 48-49°.

Anal. Found: C, 75.16; H, 5.93. Calcd. for  $C_{10}H_9NO$ :  
C, 75.47; H, 5.66.

### 3,6-Dimethylquinoline 1-oxide

The title compound was obtained in 66.0% yield using the general procedure for the preparation of N-oxides. The product had m.p. 69-71° (ethanol).

Anal. Found: C, 76.23; H, 6.72.  $C_{11}H_{11}NO$  requires:  
C, 76.30; H, 6.36.

### 3,7-Dimethylquinoline 1-oxide

The title compound was obtained in 63.3% yield using the general procedure for the preparation of N-oxides. The product had m.p. 130.5-132° (ethanol).

Anal. Found: C, 76.50; H, 6.70; N, 7.70.  $C_{11}H_{11}NO$  requires: C, 76.30; H, 6.36; N, 8.09.

### 6,7-Dimethylquinoline 1-oxide

The general procedure for the preparation of N-oxides was used and a 48.5% yield of the title compound was isolated. The product had m.p. 165-167° (water).

Anal. Found: C, 76.40; H, 6.59; N, 7.92.  $C_{11}H_{11}NO$  requires: C, 76.30; H, 6.36; N, 8.09.



#### 4-Chloroquinoline 1-oxide

##### Method A

4-Nitroquinoline 1-oxide (0.56 g) was added slowly, with ice cooling, to acetyl chloride (5 ml). The reaction mixture was kept below 40° for forty-five minutes and then ice water was added. The reaction was made basic by the addition of 10% sodium carbonate solution and extracted with chloroform. Removal of the chloroform gave the title compound (0.50 g). The product had m.p. 133-135° (acetone). Reported (Ochiai, 1953a) m.p. 133-133.5°.

##### Method B

Concentrated hydrochloric acid (5 ml) was added to 4-nitroquinoline 1-oxide (0.06 g) and the solution was boiled under reflux for one-half hour. The cooled solution was made basic with 10% sodium carbonate solution and extracted with chloroform. Removal of the chloroform gave the title compound (0.05 g).

#### 2-Chloro-4-nitroquinoline 1-oxide

2-Chloroquinoline 1-oxide (2.2 g) was dissolved in 80% sulfuric acid (12.5 ml). Potassium nitrate (2.5 g) was added and the mixture heated for three hours at 80°. The reaction mixture was added to ice water with stirring and the product which separated was filtered off. The residue was washed with water until the washings were free of acid and then with dilute sodium carbonate solution until the washings were almost free from color. A







final washing with water, and drying gave the title compound (1.2 g). The product had m.p. 153-155° (acetone). Reported (Yamazaki et al, 1968) m.p. 154-156°.

IR spectrum (Nujol mull): 1340, 1515  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

### 2-Chloroquinoline 1-oxide

#### Method A

2-Chloroquinoline (10.6 g), 30% hydrogen peroxide (7 ml) and glacial acetic acid (20 ml) were heated gently at 70° for four hours. More 30% hydrogen peroxide (5 ml) was added and the heating continued for thirteen hours. Volatile material was removed under vacuum, water (20 ml) was added and the mixture was further concentrated. Sufficient chloroform and potassium carbonate were added to form a thick paste. After standing for a few hours the mixture was filtered and the filtrate extracted with chloroform. Removal of the chloroform gave a brown oil which solidified when triturated with petroleum ether. The solid (2.2 g) had m.p. 175-180° and the IR spectrum (nujol mull) had absorption bands at 3700-2000 (OH), 1650  $\text{cm}^{-1}$  (C=O) and was identical with an authentic sample of 2(1H)-quinolone.

#### Method B (Kamiya, 1961)

A mixture of 2-chloroquinoline (5 g), monochloroacetic acid (150 g), 30% hydrogen peroxide (10 ml) and sufficient glacial acetic acid to give a homogeneous solution was kept for five days at room temperature. In-



soluble material was filtered off and the filtrate was made basic with potassium carbonate. The reaction mixture was extracted with chloroform. The chloroform was removed and the product taken up into benzene. The benzene solution was passed down an alumina column (2 1/2 cm x 10 cm) and the effluent concentrated to give starting material (2.5 g). The developing solvent was changed to benzene:methanol (50:50) and the effluent was concentrated yielding 2(1H)-quinolone (0.98 g). Increasing the methanol concentration of the developing solvent did not give any further product.

#### Method C

50% Hydrogen peroxide (2 ml) was added dropwise over twenty minutes to a mixture of 2-chloroquinoline (5 g), *n*-heptane (10 ml) and phthalic anhydride (8 g). The mixture was heated at 50° for one hour. Filtration, and evaporation of the filtrate gave recovery of starting material (1.4 g).

#### Method D (Yamazaki *et al*, 1966)

To a stirred and ice-cooled solution of maleic anhydride (42 g) in chloroform (150 ml), 30% hydrogen peroxide (8 ml) was added. After two hours stirring 2-chloroquinoline (4.8 g) was added to the mixture and the whole was kept in the refrigerator for five days. During this time maleic acid deposited and was neutralized with a small amount of concentrated potassium carbonate solu-



tion. The chloroform layer was separated, dried over potassium carbonate and then evaporated. The residue was taken up into chloroform and chromatographed on an alumina column (1 3/4 cm x 15 1/2 cm). The chloroform eluate yielded an oil which solidified on the addition of a few drops of water. The product was 2-chloroquinoline 1-oxide (3.65 g) and had m.p. 83-86° (ethanol). Reported (Yamazaki et al, 1966) m.p. 85-92° (acetone-petroleum ether).

#### Attempted preparation of 2,4-dichloro-3-phenylquinoline 1-oxide

A method similar to that described above (Method D) for the preparation of 2-chloroquinoline 1-oxide was carried out on 2,4-dichloro-3-phenylquinoline (3.2 g). A brown solid (1.6 g) was recovered and the IR spectrum was identical to that of the starting material.

#### Preparation of Hydroxamic Acids

##### Oxidation with alkaline Potassium Ferricyanide

##### General Procedure

To a vigorously stirred solution of the N-oxide in water, aqueous solutions of potassium ferricyanide and of potassium hydroxide were added separately, dropwise, at 5 to 10°, the rate of addition being regulated so that the addition of both solutions was finished at the same time (about one hour). After further stirring (one



hour) and standing overnight at room temperature, the solution was separated from the precipitate, extracted with chloroform and evaporated to give any unreacted oxide. The residual alkaline solution was acidified with acetic acid and extracted with chloroform. The chloroform solution was dried and evaporated to give the hydroxamic acid.

#### 1-Hydroxy-2(1H)-quinolone

The general procedure was employed using quinoline 1-oxide (4.5 g in 37.5 ml water), potassium ferricyanide (32.5 g in 75 ml water) and potassium hydroxide (15 g in 25 ml water) to give a 50% yield of the title compound, m.p. 187-189° (methanol). Reported (Hamana and Yamazaki, 1962) m.p. 188-191°.

IR spectrum (KBr disc): 3700-2000 (OH), 1645  $\text{cm}^{-1}$  (C=O).

#### 1-Hydroxy-4-methyl-2(1H)-quinolone

A 25% yield of the title compound was obtained using the general procedure, from 4-methylquinoline 1-oxide (2.4 g), potassium ferricyanide (15 g in 35 ml water) and potassium hydroxide (7.5 g in 12 ml water). The product had m.p. 225-226.5° (methanol). Reported (Hamana and Yamazaki, 1962) m.p. 223-225°.

IR spectrum (KBr disc): 3700-2200 (OH), 1640  $\text{cm}^{-1}$  (C=O).







## 2-Hydroxy-1(2H)-isoquinolone

Isoquinoline 1-oxide (2.5 g), potassium ferricyanide (15 g in 35 ml water) and potassium hydroxide (7.5 g in 12 ml water) were reacted according to the general procedure to give a 25% yield of the title compound, m.p. 190-193° (methanol). Reported (Hamana and Yamazaki, 1962) m.p. 185-187°.

IR spectrum (KBr disc): 3700-2200 (OH); 1632, 1612  $\text{cm}^{-1}$  (C=O).

## Oxidation with Lead Tetraacetate

### General procedure for the preparation of hydroxamic acids

A procedure similar to that described by Ohta and Ochiai (1962) was used. To a solution of the N-oxide in benzene is added lead tetraacetate and calcium carbonate. The mixture is boiled under reflux for one and one-half hours. The separated precipitate is collected by filtration and washed with chloroform. The combined filtrate and washings are concentrated under reduced pressure. The resulting residue is mixed with 10% hydrochloric acid and warmed (95-100°) for thirty minutes. The solid is removed and taken up into 10% sodium carbonate solution which is extracted with ether. Acidification of the aqueous layer, extraction of it with ether and evaporation of the ether yields the hydroxamic acid.



1-Hydroxy-4-nitro-2(1H)-quinolone

Method A

4-Nitroquinoline 1-oxide (0.4 g), lead tetraacetate (4 g) and calcium carbonate (0.1 g) were boiled under reflux in benzene (20 ml) according to the general procedure. The title compound (0.12 g) was obtained and had m.p. 198-200° (ethanol). Reported (Yamazaki et al, 1968) m.p. 205-206°.

Anal. Found: C, 52.42; H, 3.32. Calcd. for C<sub>9</sub>H<sub>6</sub>N<sub>2</sub>O<sub>4</sub>: C, 52.42; H, 2.91.

M<sup>+</sup> (mass spectrum): 206

IR spectrum (Nujol mull): 3300-2100 (OH); 1660 (C=O); 1354, 1525 cm<sup>-1</sup> (NO<sub>2</sub>).

Method B

2-Chloro-4-nitroquinoline 1-oxide (0.2 g) was heated at 98° for thirty minutes with acetic anhydride (2 ml) and sodium acetate (0.1 g). The reaction mixture was added to ice water (10 ml) and stirred. The solid which separated was collected by filtration and heated at 98° for thirty minutes with 10% hydrochloric acid solution (10 ml). The crystalline product (0.11 g) was the title compound.

IR spectrum (Nujol mull): 3300-2100 (OH); 1662 (C=O); 1354, 1525 cm<sup>-1</sup> (NO<sub>2</sub>).

Method C

An attempt to prepare the title compound was made



using the general procedure for the preparation of hydroxamic acids by oxidation with alkaline potassium ferricyanide. Only 10 mg of product was obtained from 1.8 g of 4-nitroquinoline 1-oxide. The product did not give the characteristic magenta color with ferric chloride solution and wasn't examined further.

1-Hydroxy-3-nitro-2(1H)-quinolone

3-Nitroquinoline 1-oxide (0.3 g), lead tetraacetate (2 g) and calcium carbonate (0.1 g) were boiled under reflux in benzene (20 ml) according to the general procedure to give a 61.5% yield of the title compound, m.p. 214-216° (ethanol).

Anal. Found: C, 52.54; H, 3.31:  $C_9H_6N_2O_4$  requires: C, 52.42; H, 2.91.

$M^+$  (mass spectrum): 206.

IR spectrum (Nujol mull): 3600-2100 (OH); 1650, 1615 (C=O); 1330, 1510  $cm^{-1}$  ( $NO_2$ ).

1-Hydroxy-5-nitro-2(1H)-quinolone

The general procedure for the preparation of hydroxamic acids by oxidation with lead tetraacetate was used. 5-Nitroquinoline 1-oxide (0.5 g), lead tetraacetate (5 g), and calcium carbonate (0.2 g) were boiled under reflux in benzene (30 ml) to give a 23.1% yield of the title compound, m.p. 220-223° (ethanol).

Anal. Found: C, 52.29; H, 3.04; N, 13.43.



$C_9H_6N_2O_4$  requires: C, 52.42; H, 2.91; N, 13.59.

IR spectrum (Nujol mull): 3300-2000 (OH); 1680, 1620 (C=O); 1335, 1515  $cm^{-1}$  ( $NO_2$ ).

1-Hydroxy-6-nitro-2(1H)-quinolone

6-Nitroquinoline 1-oxide (0.19 g), lead tetraacetate (1 g) and calcium carbonate (0.1 g) in benzene (20 ml) were reacted according to the general procedure to give a 46.1% yield of the title compound, m.p. 285-286°.

Anal. Found: C, 52.42; H, 2.74; N, 13.42.

$C_9H_6N_2O_4$  requires: C, 52.42; H, 2.91; N, 13.59.

IR spectrum (Nujol mull): 3700-2000 (OH); 1650, 1620 (C=O); 1340, 1525  $cm^{-1}$  ( $NO_2$ ).

1-Hydroxy-3-methyl-2(1H)-quinolone

3-Methylquinoline 1-oxide (0.5 g), lead tetraacetate (2.5 g) and calcium carbonate (0.1 g) were boiled under reflux in benzene (20 ml). The title compound was obtained in a 56.4% yield using the general procedure, m.p. 178-180° (benzene). Reported (Coutts *et al*, 1965) m.p. 182°.

IR spectrum (Nujol mull): 3500-2000 (OH); 1630  $cm^{-1}$  (C=O).

1-Hydroxy-3,6-dimethyl-2(1H)-quinolone

3,6-Dimethylquinoline 1-oxide (0.3 g), lead tetraacetate (3 g) and calcium carbonate (0.1 g) were





boiled under reflux in benzene (20 ml) according to the general procedure to give a 45.9% yield of the title compound, 172-174° (ethanol).

Anal. Found: C, 69.64; H, 5.77; N, 7.06.

$C_{11}H_{11}NO_2$  requires: C, 69.84; H, 5.82; N, 7.41.

IR spectrum (Nujol mull): 3600-2100 (OH); 1632  $cm^{-1}$  (C=O).

#### 1-Hydroxy-3,7-dimethyl-2(1H)-quinolone

The general procedure for the preparation of hydroxamic acids was employed, using 3,7-dimethylquinoline 1-oxide (1 g), lead tetraacetate (5 g), calcium carbonate (0.2 g) and benzene (50 ml). The title compound was obtained in a 45.8% yield, m.p. 169-170° (ethanol).

Anal. Found: C, 69.45; H, 5.47; N, 7.24.

$C_{11}H_{11}NO_2$  requires: C, 69.84; H, 5.82; N, 7.41.

IR spectrum (Nujol mull): 3300-2000 (OH); 1630  $cm^{-1}$  (C=O).

#### 1-Hydroxy-6,7-dimethyl-2(1H)-quinolone

6,7-Dimethylquinoline 1-oxide (0.5 g), lead tetraacetate (2.5 g), calcium carbonate (0.1 g) and benzene (20 ml) were reacted according to the general procedure to give a 48.9% yield of the hydroxamic acid, m.p. 234-235° (ethanol); 228-230° (benzene).

Anal. Found: C, 69.65; H, 6.01; N, 7.54.

$C_{11}H_{11}NO_2$  requires: C, 69.84; H, 5.82; N, 7.41.



IR spectrum (Nujol mull): 3500-2000 (OH); 1630  $\text{cm}^{-1}$  (C=O) .

#### 4-Chloro-1-hydroxy-2(1H)-quinolone

##### Method A

The general procedure was employed using 4-chloro-quinoline 1-oxide (0.4 g), lead tetraacetate (2 g), calcium carbonate (0.1 g) and benzene (20 ml). The title compound was obtained in a 27.5% yield, m.p. 198-199 $^{\circ}$  (ethanol). Reported (Ochiai and Ohta, 1962) m.p. 200-202 $^{\circ}$ .

IR spectrum (Nujol mull): 3500-2000 (OH); 1670, 1630  $\text{cm}^{-1}$  (C=O) .

##### Method B

1-Acetoxy-4-chloro-2(1H)-quinolone (0.01 g) was heated on a water bath for one-half hour with 10% hydrochloric acid solution (5 ml). The mixture was then taken up into 10% sodium carbonate solution and reacidified with hydrochloric acid. Extraction of the acidic solution with chloroform, and evaporation, yielded the title compound (0.005 g).

#### 1-Acetoxy-4-chloro-2(1H)-quinolone

2-Chloro-4-nitroquinoline 1-oxide (1.5 g) was boiled under reflux with acetyl chloride (5 ml) for thirty minutes. The mixture was added to ice water with stirring. The precipitated product was collected



by filtration. A 52.9% yield of the title compound was obtained, m.p. 95-96°. Reported (Yamazaki et al, 1968) m.p. 101-103°.

IR spectrum (Nujol mull): 1800 (NOAc); 1662  $\text{cm}^{-1}$  (C=O).

### Reductive Cyclization with Sodium Borohydride and palladium-Charcoal

#### General method of preparing hydroxamic acids by reducing esters with sodium borohydride and palladium-charcoal

Sodium borohydride (1.5 g) was dissolved in water (5 ml) and palladium-charcoal (0.1 g) suspended in water (5 ml) was carefully added. The suspension was diluted with dioxane (10 ml) and nitrogen was passed through the mixture while the ester (1-2 g) in dioxane (10-20 ml) was added dropwise over a period of thirty minutes. The passage of nitrogen was continued for thirty minutes after the ester had been added. The mixture was then filtered, acidified and diluted with water. In some cases the hydroxamic acid precipitated and was filtered off. In other cases, the solution was concentrated and extracted with ether. Evaporation of the ether yielded the hydroxamic acid.

Ethyl 1-hydroxy-2(1H)-quinolone-3-carboxylate (Coutts, 1969)

#### Method A

Ethyl o-nitrobenzylidenemalonate (2 g) was re-



duced according to the general procedure to give a 63.5% yield of the title compound, m.p. 163-165° (ethanol). Reported (Coutts, 1969) m.p. 167-169°.

IR spectrum (Nujol mull): 3300-2000 (OH); 1732 (ester C=O); 1620  $\text{cm}^{-1}$  (hydroxamate C=O).

Concentration of the filtrate, after removal of the title compound, to near dryness gave a product which was taken up into ether. The ether solution was extracted with 10% sodium carbonate solution and the aqueous layer was acidified with dilute hydrochloric acid. Extraction of the aqueous layer with ether and evaporation yielded ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate (0.05 g), m.p. 134-135°. Reported (Coutts, 1969) m.p. 135-137°; (Coutts, Noble and Wiberley, 1964) m.p. 137-139°.

IR spectrum (Nujol mull); 3300-2000 (OH); 1730 (ester C=O); 1670, 1640  $\text{cm}^{-1}$  (hydroxamate C=O).

#### Method B

Ethyl o-nitrobenzylidenemalonate (2 g) was reduced according to the general procedure except that methanol was the solvent. The title compound was obtained in a 25.2% yield, m.p. 163-165°.

#### 1-Hydroxy-2(1H)-quinolone-3-carboxylic acid

Ethyl 1-hydroxy-2(1H)-quinolone-3-carboxylate (0.2 g) was boiled under reflux, for two hours, in 5N sodium hydroxide solution (5 ml). The reaction mixture





was cooled, acidified and the product was collected by filtration. A 57.1% yield of the title compound was obtained, m.p. 259-260°. Reported (Coutts, 1969) m.p. 259-261°; (Coutts and Wibberley, 1963) m.p. 258-259°.

IR spectrum (Nujol mull): 3220 (OH); 1740 (acid C=O); 1628  $\text{cm}^{-1}$  (hydroxamate C=O).

### 1-Hydroxy-3-methyl-2(1H)-quinolone

#### Method A

Ethyl  $\beta$ -o-nitrophenyl- $\alpha$ -methylacrylate (1 g) was reduced according to the general procedure. The filtrate was extracted with ether and the resulting ether solution extracted with 10% sodium carbonate solution. The aqueous layer was then acidified and extracted with ether. Evaporation of the ether solution yielded the title compound (15 mg), m.p. 180-182° (benzene). Reported (Coutts et al, 1965) m.p. 182°.

IR spectrum (Nujol mull): 3500-2000 (OH); 1630  $\text{cm}^{-1}$  (C=O).

#### Method B

The same procedure (Method A) was repeated except that a solution of ethyl  $\beta$ -o-nitrophenyl- $\alpha$ -methylacrylate (1 g) in dioxane was heated prior to its addition to the suspension of sodium borohydride and palladium-charcoal. A low yield of the title compound was obtained (18 mg).



Method C

A solution of ethyl  $\beta$  -*o*-nitrophenyl- $\alpha$ -methyl-acrylate (1 g) in dioxane (30 ml) was stirred for four hours during which time an ultra-violet light was shone on the solution (Blak-Ray B-100A Long Wave U.V. Lamp). The reduction was then carried out as in Method A. 20 mg of the title compound were obtained.

3,4-Dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine

Methyl  $\alpha$  -(*o*-nitrophenylthio)propionate (1.5 g) was reduced according to the general procedure. The title compound (0.3 g) was obtained, m.p. 144-147°. Reported (Coutts, Peel and Smith, 1965) m.p. 149-150°.

IR spectrum (Nujol mull): 3200 (OH); 1655 cm<sup>-1</sup> (C=O).

3,4-Dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine 1,1-dioxide

Methyl  $\alpha$  -(*o*-nitrobenzenesulfonyl)propionate (0.5 g) was reduced according to the general procedure to give the title compound (0.4 g), m.p. 189-191°. Reported (Coutts, Peel and Smith, 1965) m.p. 186-188°.

IR spectrum (Nujol mull): 3250 (OH); 1660 cm<sup>-1</sup> (C=O).

3,4-Dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide

Methyl  $\alpha$  -(*o*-nitrobenzenesulfonyl)acetate (2 g) was reduced according to the general procedure to give the



title compound (1 g), m.p. 149-150°. Reported (Coutts and Wibberley, 1963) m.p. 149-150°.

IR spectrum (Nujol mull): 3600-2300 (OH); 1655  $\text{cm}^{-1}$  (C=O).

2-Bromo-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide

To a solution of 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (1.9 g) in glacial acetic acid (50 ml) was added pyridinium bromide perbromide (3 g) over a one-half hour period, with stirring. The stirring was continued for twelve hours and then water (200 ml) was added. The aqueous solution was extracted with ether and evaporated to yield 86.5% of the title compound, m.p. 195-197°. Reported (Brewster, 1968) m.p. 195-197°.

IR spectrum (Nujol mull): 3350 (OH); 1670  $\text{cm}^{-1}$  (C=O).

NMR spectrum ( $\text{DMSO-d}_6$ ): 1-proton singlet at  $\tau$  3.01 for  $-\overset{|}{\text{CH}}-\text{Br}$ . A complex signal between  $\tau$  1.8 and 2.6 integrated for 5 protons (N-OH and aromatic).

2-n-Butyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine

The general procedure for the preparation of cyclic hydroxamic acids with sodium borohydride and palladium-charcoal was employed using ethyl  $\alpha$ -(o-nitrophenoxy) hexanoate (2 g). The title compound (0.2 g) was obtained on acidification of the filtrate with acetic acid. The product had m.p. 96-97° (ethanol). Reported (Coutts and



Hindmarsh, 1966) m.p. 97-99°.

IR spectrum (Nujol mull): 3100 (OH), 3600-2400 (OH); 1680, 1650  $\text{cm}^{-1}$  (C=O).

2-Ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine

The title compound (0.1 g) was obtained by the reduction of ethyl  $\alpha$ -(*o*-nitrophenoxy)butyrate (2 g) according to the general procedure. The product had m.p. 133-135°. Reported (Coutts and Hindmarsh, 1966) m.p. 133-135°.

IR spectrum (KBr disc): 3400-2400 (OH); 1670, 1635  $\text{cm}^{-1}$  (C=O).

7-Chloro-2-ethyl-3,4-dihydro-3-oxo-2H-1,4-benzoxazine

2-Ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (10 mg) was dissolved in sodium carbonate solution (5 ml). Acidification with hydrochloric acid, and allowing the solution to stand for two days yielded a solid (5 mg) which was collected by filtration. This product did not give the characteristic magenta color on the addition of ferric chloride solution and was identified as the title compound. The product had m.p. 143-144°.

Anal. Found: N, 6.30.  $\text{C}_{10}\text{H}_{10}\text{NO}_2\text{Cl}$  requires: N, 6.62.

$\underline{\text{M}}^+$  (mass spectrum): 211 (67%), 213 (19.7%).

IR spectrum (Nujol mull): 3190 (NH); 1696  $\text{cm}^{-1}$  (C=O).







Preparation of the Intermediates Used  
for Reductive Cyclization

Ethyl o-nitrobenzylidenemalonate (Loudon and Wellings, 1960)

o-Nitrobenzaldehyde (4.5 g), diethylmalonate (6 g), acetic anhydride (4.8 g) and potassium bicarbonate (4.5 g) were placed in a flask and heated at 98° for three hours. The reaction mixture was cooled, poured into water (150 ml) and extracted with ether. A yellow oil (4 g) was obtained on evaporation of the ether which solidified on standing, m.p. 45-47°. Reported (Loudon and Wellings, 1960) m.p. 53°.

IR spectrum (thin film): 1730 (C=O); 1345, 1525  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

$\beta$ -o-Nitrophenyl- $\alpha$ -methylacrylic acid (Cunningham et al, 1949)

A mixture of o-nitrobenzaldehyde (10.1 g), propionic anhydride (14 ml) and anhydrous sodium propionate (6.4 g) was heated at 150° for twenty one hours. The reaction mixture was poured into water and the solid which precipitated was collected by filtration. The residue was taken up into dilute alkali and acidification with hydrochloric acid yielded the title compound (7.7 g), m.p. 195-198° (ethanol). Reported (Cunningham et al, 1949) m.p. 194°.

IR spectrum (Nujol mull): 3700-2200 (OH); 1695



(C=O) : 1340, 1525  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Ethyl  $\beta$ -o-nitrophenyl- $\alpha$ -methylacrylate

A solution of  $\beta$ -o-nitrophenyl- $\alpha$ -methylacrylic acid (4.2 g) in anhydrous ethanol (50 ml) and concentrated sulfuric acid (2 ml) was boiled under reflux for twenty six hours. The mixture was then concentrated under reduced pressure to give a brown oil which was mixed with ice water and neutralized with a concentrated solution of sodium carbonate. Extraction of the aqueous layer with ether and evaporation yielded an oil which solidified on standing (4.2 g). The product had m.p. 59-61° (ethanol). Reported (Cunningham et al, 1949) m.p. 60°.

IR spectrum (Nujol mull): 1710 (C=O); 1345, 1520  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Methyl  $\alpha$ -(o-nitrobenzenesulfonyl)propionate

To a solution of methyl  $\alpha$ -(o-nitrophenylthio) propionate (1.1 g) in glacial acetic (20 ml) was added potassium permanganate (2 g) in water (5 ml). The whole was stirred at room temperature for six and three-quarter hours. Sufficient 30% hydrogen peroxide solution was added to dissolve the manganese dioxide that formed and to decolorize the solution. The solution was neutralized by the addition of sodium carbonate, flooded with water and extracted with ether. Evaporation of the ether yielded a viscous oil which solidified on the



addition of a small amount of water. The title compound (0.84 g) had m.p. 88-90°. Reported (Coutts, Peel and Smith, 1965) m.p. 87-88°.

IR spectrum (KBr disc): 1752 (C=O); 1328, 1542  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

(o-Nitrobenzenethio)acetic acid (Badger *et al*, 1957)

o-Nitrochlorobenzene (96 g), thioglycollic acid (45.6 g) and sodium bicarbonate (120 g) were dissolved in 50% ethanol (440 ml) and boiled under reflux for three and one-half hours. Ethanol (200 ml) was distilled and water (150 ml) was added. The hot solution was filtered and the filtrate acidified with dilute hydrochloric acid. A thick yellow suspension formed and was filtered. The residue (76.2 g) was the title compound and had m.p. 159-160°. Reported (Badger *et al*, 1959) m.p. 164°.

IR spectrum (Nujol mull): 3400-2000 (OH); 1330, 1560  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Methyl  $\alpha$ -(o-nitrobenzenethio)acetate

A solution of (o-nitrobenzenethio)acetic acid (76.2 g) in methanol (800 ml) and concentrated sulfuric acid (72 ml) was boiled under reflux for six and one-half hours. Crystals formed when the solution was allowed to cool. These crystals were collected by filtration and the filtrate was concentrated yielding further product. The title compound (77 g) had m.p. 88-90°.



Reported (Coutts and Wibberley, 1963) m.p. 88-90°.

IR spectrum (Nujol mull): 1725 (C=O); 1330, 1560  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Methyl  $\alpha$ -(o-nitrobenzenesulfonyl)acetate

Methyl  $\alpha$ -(o-nitrobenzenethio)acetate (10 g) was dissolved in glacial acetic acid (150 ml) and to the well stirred solution was added a solution of potassium permanganate (20 g) in water (10 ml). The reaction was stirred for five and one-half hours. Sufficient 30% hydrogen peroxide solution was added slowly until the mixture became colorless. A precipitate formed which was collected by filtration. The yield of the title compound was increased by extracting the filtrate with ether. Total weight of the title compound was 10.6 g and the product had m.p. 119-120°. Reported (Coutts and Smith, 1967) m.p. 120-121°.

IR spectrum (Nujol mull): 1750 (C=O); 1325, 1530  $\text{cm}^{-1}$  ( $\text{NO}_2$ ).

Ethyl  $\alpha$ -(o-nitrophenoxy)hexanoate (Coutts and Hindmarsh, 1966)

The sodium salt of o-nitrophenol (8.1 g) was suspended in acetone (100 ml) and ethyl 2-bromohexanoate (11.2 g) was added. The mixture was boiled under reflux for seven days. The unreacted starting material and sodium bromide were removed by filtration, and the crude product was obtained from the filtrate by distil-







ling off the acetone. The oily product (5 g) solidified on standing and was not further purified before reduction.

Ethyl  $\alpha$ -(o-nitrophenoxy)butyrate (Coutts and Hindmarsh, 1966)

To the sodium salt of o-nitrophenol (4 g), suspended in acetone (50 ml) was added ethyl 2-bromobutyrate (3.7 ml). The mixture was boiled under reflux for fifty hours. The unreacted starting material and sodium bromide were removed by filtration. Evaporation of the filtrate yielded the title compound (2.0 g) which was immediately reduced with sodium borohydride and palladium-charcoal.

### Attempted Preparation of Thiohydroxamic Acids

#### General procedure

The hydroxamic acid is boiled under reflux in dry toluene (20 ml), with stirring, with phosphorous pentasulfide for one-half hour. The hot solution is filtered and the residue washed with hot toluene. The yield is increased by evaporating the filtrate, stirring the residue with water and collecting the water insoluble material by filtration.

#### Reaction of phosphorous pentasulfide with 1-hydroxy-2(1H)-quinolone

1-Hydroxy-2(1H)-quinolone (1 g) and phosphorous



pentasulfide (1 g) were reacted according to the general procedure to yield a yellow solid (0.74 g), m.p. 170-173°. The product did not give any coloration on the addition of alcoholic ferric chloride solution. The IR spectrum (KBr disc) showed a strong absorption band at  $1104\text{ cm}^{-1}$  (C=S), and was identical to the IR spectrum of 2-mercaptoquinoline.

### 2-Mercaptoquinoline

2(1H)-Quinolone (1 g) and phosphorous pentasulfide (0.6 g) were reacted according to the general procedure. The title compound was obtained in a 59.4% yield, m.p. 175°. Reported (Roos, 1888) m.p. 174°; (Albert and Barlin, 1959) m.p. 178-179.5°.

IR spectrum (KBr disc):  $1104\text{ cm}^{-1}$  (C=S).

### Reaction of phosphorous pentasulfide with 2-hydroxy-1(2H)-isoquinolone

The general procedure was employed using 2-hydroxy-1(2H)-isoquinolone (0.5 g) and phosphorous pentasulfide (0.5 g). A solid was obtained (0.47 g) which did not give any color change on the addition of ferric chloride solution. The product had m.p. 167-168°. The reported m.p. for 1-mercaptoisoquinoline (Albert and Barlin, 1959) is 171°. The IR spectrum showed absorption peaks at 3150 (NH) and 1170 (C=S) which is similar to the absorption peaks described (Spinner, 1960) for 1-mercaptoisoquinoline.



Reaction of phosphorous pentasulfide with 1-hydroxy-4-methyl-2(1H)-quinolone

1-Hydroxy-4-methyl-2(1H)-quinolone (0.3 g) and phosphorous pentasulfide (0.4 g) were reacted according to the general procedure. The solid product obtained (0.3 g) had m.p. 258-260° (ethanol). The product did not give any color change on the addition of alcoholic ferric chloride solution. The IR spectrum had a strong absorption band at 1108  $\text{cm}^{-1}$  (C=S) and the product was assumed to be 4-methyl-2-mercaptoquinoline. Reported m.p. for 4-methyl-2-mercaptoquinoline is 253° (Roos, 1888).

Reaction of phosphorous pentasulfide with methyl (3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazin-2-yl)acetate

Methyl (3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazin-2-yl)acetate (1.1 g) was reacted with phosphorous pentasulfide (1 g) according to the general procedure. An oily substance (0.74 g) was obtained which partially solidified on triturating with ethanol and the solid was collected by filtration. The product had m.p. 257-258°. The IR spectrum had absorption bands at 3180 (NH) and a broad band from 1740-1670  $\text{cm}^{-1}$  (ester and lactam C=O).  $\text{M}^+$  (mass spectrum): 253 indicates the formation of methyl (3,4-dihydro-3-oxo-2H-1,4-benzothiazine)  $\Delta^{2,\alpha}$  acetate. Reported (Kalbag et al, 1967) m.p. 264-266°. An authentic sample of this benzothiazine gave an IR spectrum identical with that obtained



from the product of this reaction.

The remaining oil that was left after the first product was isolated was stirred with 10% sodium carbonate solution. An insoluble precipitate was obtained (0.19 g) and had m.p. 138-140° (methanol). The IR spectrum showed absorption peaks at 3200 (NH); 1750 (ester C=O) and 1675 cm<sup>-1</sup> (lactam C=O). This product was tentatively identified as methyl (3,4-dihydro-3-oxo-2H-1,4-benzothiazin-2-yl)acetate. Reported (Bourdais, 1962) m.p. 145-146°.

Acidification of the sodium carbonate solution and extraction with ether yielded, on evaporation, the starting hydroxamic acid (0.2 g).

Methyl (3,4-dihydro-3-oxo-2H-1,4-benzothiazine)  $\Delta$  2,  $\alpha$   
acetate

A procedure similar to that described by Mushkalo and Brezemska (1952) was used in which ether solutions of 2-aminothiophenol (4 g) and dimethylacetylenedicarboxylate (4 g) were mixed. An immediate precipitate was obtained and collected by filtration (2.5 g). The product had m.p. 258-261°. Reported (Kalbag et al, 1967) m.p. 264-266°.

IR spectrum (KBr disc): 3180 (NH); 1740-1670 cm<sup>-1</sup> (ester and lactam C=O).

1-Benzoyloxy-2(1H)-quinolone

The sodium salt of 1-hydroxy-2(1H)-quinolone (0.9 g)







and benzyl chloride (0.75 g) were boiled under reflux in acetone for two hours. The reaction mixture was added to ice-water and a precipitate formed which was collected by filtration (0.1 g), m.p. 104-105° (ether).

Reported (Paquette, 1966) m.p. 104-104.5°.

IR spectrum (KBr disc): 1665  $\text{cm}^{-1}$  (C=O).

### Reaction of 1-benzyloxy-2(1H)-quinolone with phosphorous pentasulfide

#### Method A

1-Benzyloxy-2(1H)-quinolone (0.1 g) and phosphorous pentasulfide (0.1 g) were reacted according to the general procedure except for refluxing for an extended period (42 hours). A solid product was obtained (0.02 g) which had an identical IR spectrum to that of 2-mercaptoquinoline. The same product was obtained when the reflux time was reduced to two hours.

#### Method B

1-Benzyloxy-2(1H)-quinolone (0.25 g) and phosphorous pentasulfide (0.25 g) were boiled under reflux for twenty four hours in pyridine (5 ml). The reaction mixture was poured into hot water (20 ml). An oil separated which crystallized over a period of three hours. The solid (0.12 g) was filtered off and washed with water. The IR spectrum indicated that starting material was recovered. However, when the reflux time was increased to fifty five hours the product obtained was 2-mercapto-



quinoline (0.21 g).

#### Method C

1-Benzyloxy-2(1H)-quinolone (0.1 g) and phosphorous pentasulfide (0.1 g) were boiled under reflux for four hours in benzene (10 ml). Filtration of the cooled reaction yielded a solid product (0.06 g) which had an identical IR spectrum to 2-mercaptoquinoline.

#### Reaction of 2-chloroquinoline 1-oxide with sodium sulfhydrate

#### Method A

2-Chloroquinoline 1-oxide (1 g) was dissolved in alcohol (20 ml). This solution was slowly added to a solution of sodium sulfhydrate (2.4 g) in water (40 ml). The mixture was allowed to stand at room temperature for one hour and then extracted with ether. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with ether. Evaporation of the ether yielded a solid (0.53 g), m.p. 67-69°. This product gave a deep blue color with ferric chloride solution and the IR spectrum showed a strong absorption peak at  $1180\text{ cm}^{-1}$  which could be due to C=S stretching. The product was believed to be 2-mercaptoquinoline 1-oxide.

Anal. Found: C, 60.78; H, 3.53; N, 7.50. Calcd. for  $\text{C}_9\text{H}_7\text{NOS}$ : C, 61.02; H, 3.95; N, 7.91.



### Method B

2-Chloroquinoline 1-oxide (0.3 g) in water (40 ml) was heated to 95°. A warm solution of sodium sulfide ( $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ ) (0.2 g) and sodium sulfhydrate (0.1 g) in water (40 ml) was added over a ten minute period. The reaction mixture was heated for forty-five minutes (95°) and cooled. The mixture was extracted with chloroform and the aqueous layer acidified with concentrated hydrochloric acid and extracted with chloroform. Evaporation of the chloroform layer yielded an oil which solidified on the addition of a few drops of water. The product (0.14 g) had m.p. 65-67.5°. The IR spectrum was identical to that obtained in Method A.

### Reaction of 2-Chloroquinoline 1-oxide with thiourea

2-Chloroquinoline 1-oxide (1 g) and thiourea (0.45 g) were boiled under reflux for seventeen hours in absolute ethanol (10 ml). The reaction mixture was cooled and concentrated to a small volume. The oily material was heated (95-98°) with 10% sodium hydroxide solution for two hours. Filtration and acidification of the filtrate yielded a solid product (0.35 g) which had an IR spectrum identical to that of 2-mercaptoquinoline.



## PART II

### MASS SPECTROMETRY





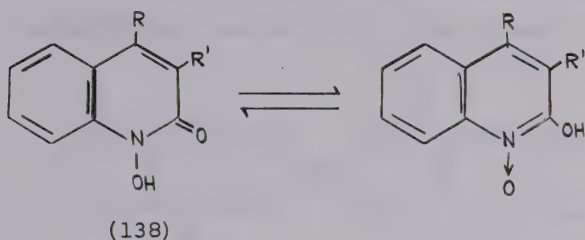
## MASS SPECTROMETRY

There has been no detailed study on the effect of electron-impact on cyclic hydroxamic acids, although in a few instances cyclic hydroxamate structures have been proposed for compounds because of the presence of an  $(M-16)^+$  ion in their spectra. Two examples are: 5,6-dimethoxy-1-hydroxy-2-oxoisoquinoline (60) (Bryce and Maxwell, 1965) and 3-(o-acetamidophenyl)-1-hydroxy-2(1H)-quinolone (9a) (Abramovitch, Coutts and Pound, 1967). The mass spectra of various N-oxides have been recorded; they include quinoline and isoquinoline N-oxides (Coutts, 1968; Bryce and Maxwell, 1965), pyridine N-oxides (Grigg and Odell, 1966; Bild and Hesse, 1967), benzimidazole N-oxides and quinoxaline N,N-dioxides (Tatemutsu et al, 1967), and phenazine mono- and di-N-oxides (Morita, 1966). In all of these studies it was observed that aromatic N-oxides gave large  $(M-16)^+$  fragment ions.

Because cyclic hydroxamic acids are o-hydroxy-N-oxides it might be expected that they could be characterized by the production of abundant  $(M-16)^+$  and  $(M-17)^+$  ions. In this present study the following hydroxamic acids were subjected to electron impact to observe whether or not they fragmented predictably and to see if cyclic hydroxamic acids could be characterized by their  $(M-16)^+$  and  $(M-17)^+$  ions; the spectra of 1-hydroxy-2(1H)-quinolone (21), 1-hydroxy-4-methyl-2(1H)-

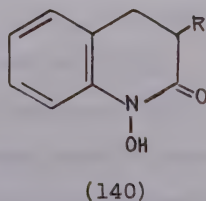
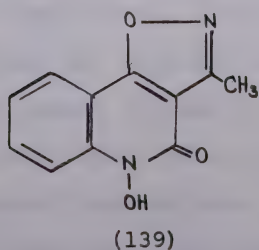


quinolone (41), 3-cyano-1-hydroxy-2(1H)-quinolone (138a), 3-amino-1-hydroxy-2(1H)-quinolone (61), 1-hydroxy-2(1H)-



	R	R'		R	R'
21	H	H	138b	H	COOH
41	Me	H	9a	H	<u>o</u> -PhNHCOMe
138a	H	CN	138c	OH	H
61	H	NH <sub>2</sub>			

quinolone-3-carboxylic acid (138b), 3-(o-acetamidophenyl)-1-hydroxy-2(1H)-quinolone (9a) and 1,4-dihydroxy-2(1H)-quinolone (138c) were recorded and examined. Other hydroxamic acids studied included 4,5-dihydro-5-hydroxy-3-methyl-4-oxoisoxazolo-(4,5-c)-quinoline (139), ethyl

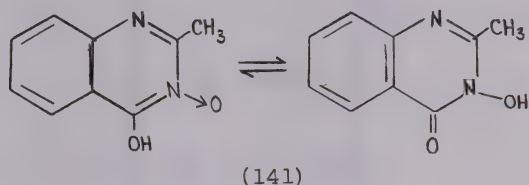


140) R=CN

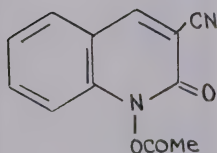
128) R=COOEt



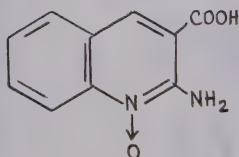
3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate (128), 3-cyano-3,4-dihydro-1-hydroxy-2(1H)-quinolone (140), 2-hydroxy-1(2H)-isoquinolone (40) and 4-hydroxy-2-methylquinazoline-3-oxide (141). Two closely related



compounds, 1-acetoxy-3-cyano-2(1H)-quinolone (142) and 2-aminoquinoline-3-carboxylic acid 1-oxide (143) were



(142)



(143)

also investigated. The mass spectra of most of these compounds are included in Figures 2 to 6. All compounds except 3-cyano-3,4-dihydro-1-hydroxy-2(1H)-quinolone (140), ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate (128) and 1-acetoxy-3-cyano-2(1H)-quinolone (142) can exist in the fully aromatic



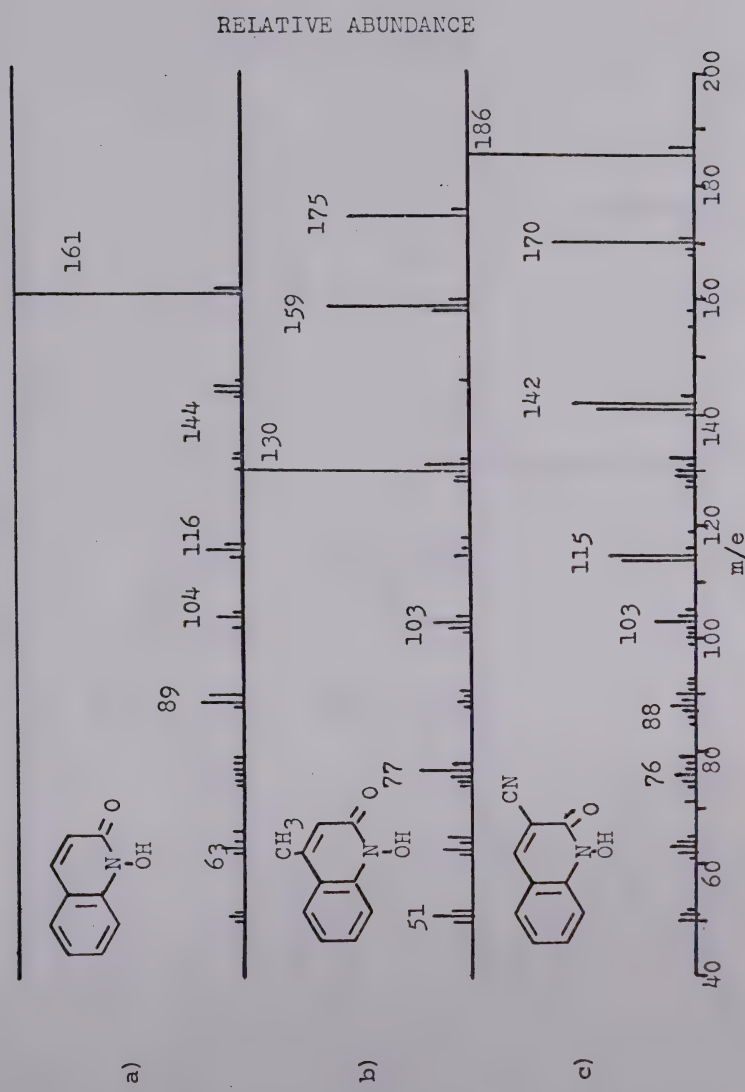


Figure 2: Mass spectra of a) 1-hydroxy-2(1H)-quinolone, b) 1-hydroxy-4-methyl-2(1H)-quinolone and c) 3-cyano-1-hydroxy-2(1H)-quinolone.





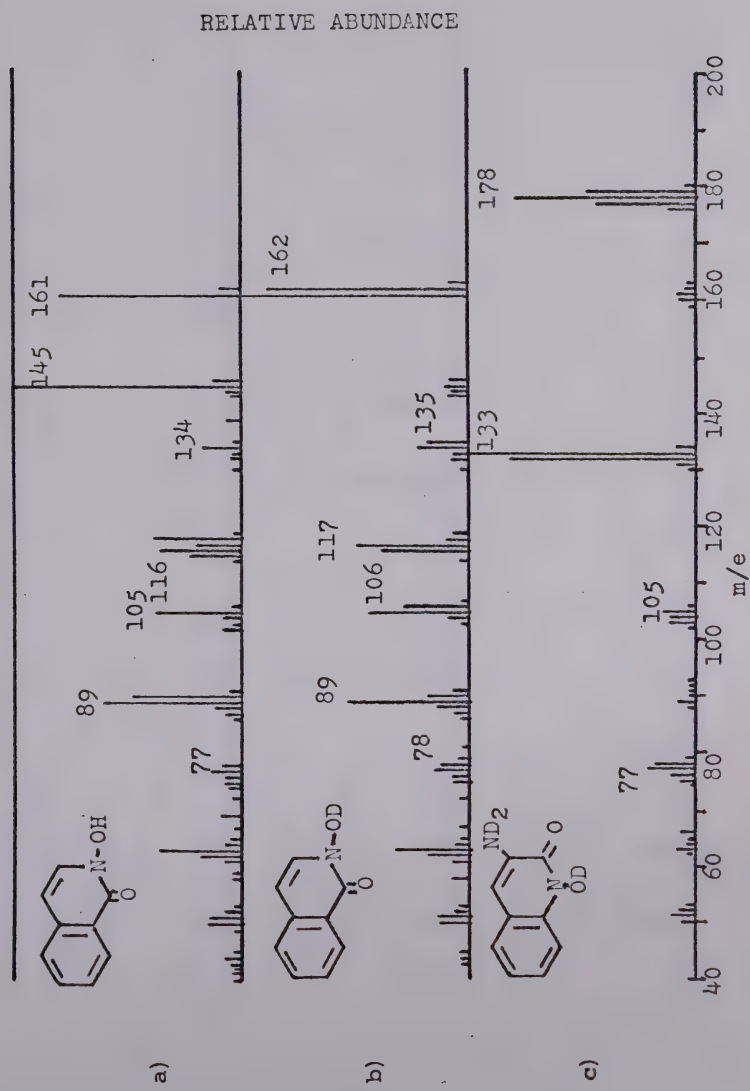


Figure 3: Mass spectra of a) 2-hydroxy-1(2H)-isoquinoline, b) 2-hydroxy-d-1(2H)-isoquinoline and c) 3-amino-d<sub>2</sub>-1-hydroxy-d-2(1H)-quinoline.





Figure 4: Mass spectra of 3-amino-1-hydroxy-2(1H)-quinolone b) 3-cyano-3,4-dihydro-1-hydroxy-2(1H)-quinolone and c) 1,4-dihydroxy-2(1H)-quinolone.



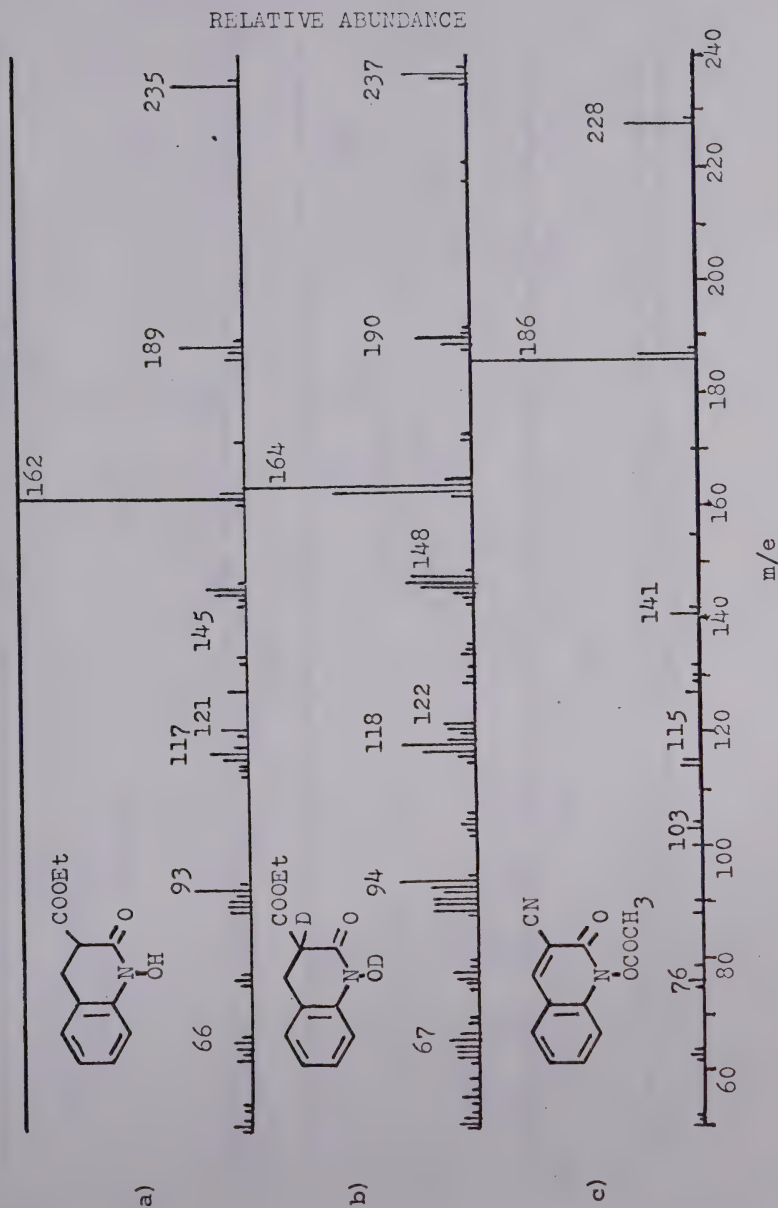


Figure 5: Mass spectra of a) ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate b) ethyl 3,4-dihydro-3-d-1-hydroxy-3-d-2(1H)-quinolone-3-carboxylate and c) 1-acetoxy-3-cyano-2(1H)-quinolone.



## RELATIVE ABUNDANCE

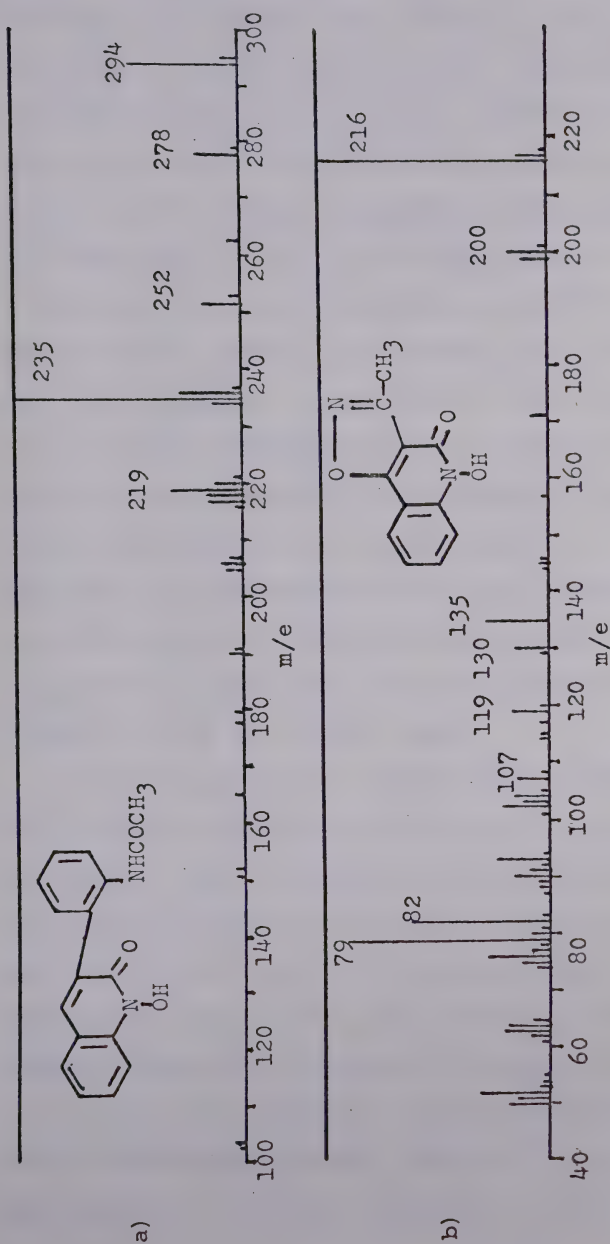


Figure 6: Mass spectra of a) 3-(o-acetamidophenyl)-1-hydroxy-2(1H)-quinolone and b) 4,5-dihydro-5-hydroxy-3-methyl-4-oxoisoxazolo-(4,5-c)-quinoline.





N-oxide tautomeric form and each would be expected to give rise to an  $(M-16)^+$  ion. Table II shows that the intensity of the  $(M-16)^+$  ion varied considerably with the compound being investigated. Particularly noticeable is the relatively low abundance (1.4%) of the  $(M-16)^+$  ion for ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone (128). This low abundance can be explained by the ease with which the hydroxamic acid loses a COOEt radical. Table II also reveals that an  $(M-17)^+$  ion is not abundant in every case. That the  $(M-17)^+$  ion is a direct fragmentation of the  $(M)^+ \rightarrow (M-OH)^+$  transition was inferred by the presence of metastable ions of appropriate mass in each spectrum. One can conclude from these observations that, contrary to what was expected, cyclic hydroxamic acids can not be characterized by the production, by direct fragmentation of abundant  $(M-16)^+$  and  $(M-17)^+$  ions.

Clugston and MacLean (1966) have observed that 2-hydroxyquinoline exhibits relatively strong peaks for the fragment ions corresponding to the loss of 28 mass units (CO) followed by the loss of 27 mass units (HCN) from the molecular ion. The mass spectrum of 2-hydroxyquinoline 1-oxide, the tautomer of 1-hydroxy-2(1H)-quinolone (21) fragments initially by the loss of an O atom and a hydroxyl radical and gives fragment ions of approximately equal intensity (9.6 and 9.9% respectively). After the loss of an O atom the  $(M-16)^+$



Table II. Relative Abundance of  $M^+$ ,  $(M-16)^+$  and  $(M-17)^+$   
Ions and Location of Base Peaks in the  
Spectra of Quinoline Hydroxamic Acids and  
Related Compounds

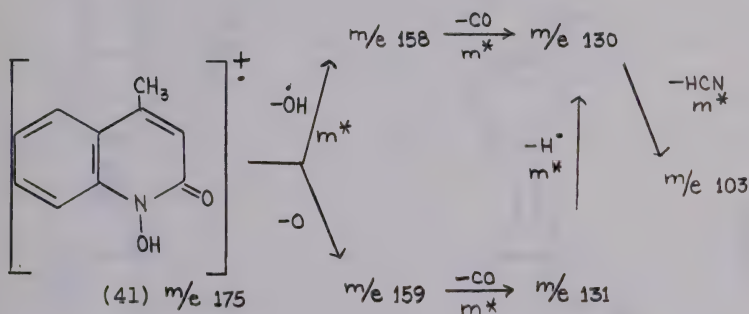
<u>Compound</u>	<u>% Relative Abundance</u>			<u>Base Peak</u>
	<u><math>M^+</math></u>	<u><math>M-16^+</math></u>	<u><math>M-17^+</math></u>	
21	100	9.6	9.9	$M^+$
41	50.3	62.0	13.9	$(M-45)^+$
138a	100	63.4	2.8	$M^+$
61	100	81.0	9.1	$M^+$
138b	36.3	7.8	12.6*	$(M-18)^+$
9a	50.2	18.8	1.2	$(M-59)^+$
138c	76.8	5.0	0.5	$(M-98)^+$
139	100	17.5	11.0	$M^+$
128	30.0	1.4	0.3	$(M-73)^+$
140	100	56.8	27.3	$M^+$
142	31.4	-	-	$(M-42)^+$
40	79.2	100	6.9	$(M-16)^+$
143	72.1	91.8	6.8*	m/e 18
141	100	12.3	7.8	$M^+$

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\*Abundance due mainly to  $(M+1-18)^+$  ion.



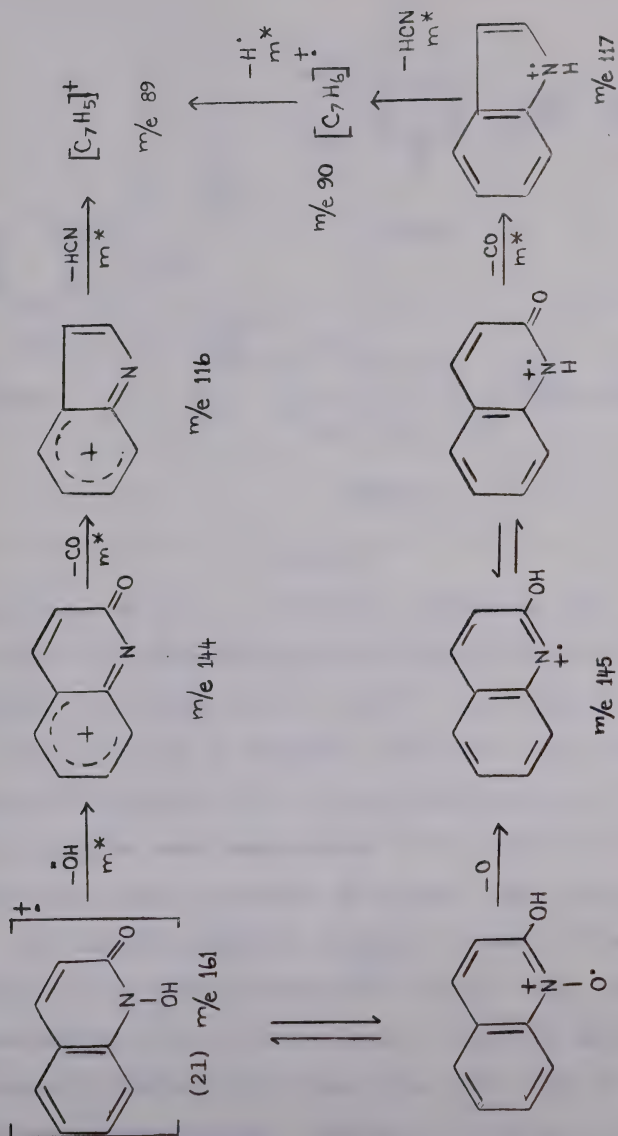
ion fragments further exactly as described for 2-hydroxy-quinoline. The  $(M-17)^+$  ion fragments similarly (Scheme 4). The mass spectrum of a closely related cyclic hydroxamic acid, 1-hydroxy-4-methyl-2(1H)-quinolone (41) is very similar (Scheme 5) to that of 1-hydroxy-2(1H)-quinolone as is the spectrum of 3-cyano-1-hydroxy-2(1H)-quinolone (138a). The latter compound expels the



Scheme 5

two nitrogen atoms as HCN molecules in two successive steps from the ions  $m/e$  142 and 141 (Scheme 6). The mass spectrum of the acetate, 1-acetoxy-3-cyano-2(1H)-quinolone (142) was also examined. After the initial loss of a ketene molecule from the molecular ion the resulting ion  $m/e$  186 fragmented exactly as shown for 3-cyano-1-hydroxy-2(1H)-quinolone in Scheme 6.

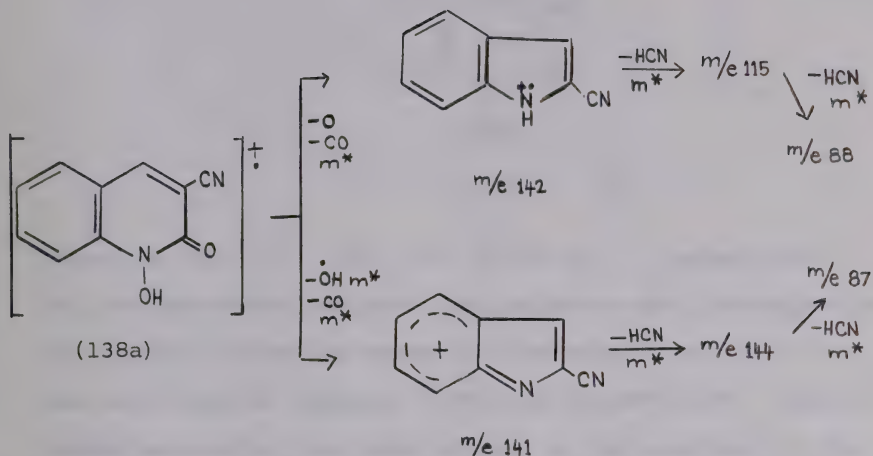




Scheme 4



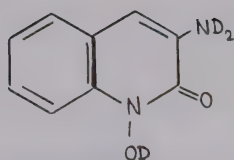




Scheme 6

A large (M-16)<sup>+</sup> ion (relative abundance 81%) was observed in the mass spectrum of 3-amino-1-hydroxy-2(1H)-quinolone (61) along with an (M-17)<sup>+</sup> ion (relative abundance 9.1%). The major fragment ions from this hydroxamic acid can be accounted for by the pathway depicted by Scheme 7. An accurate mass measurement of the ion m/e 132 revealed that it was a mixture of C<sub>8</sub>H<sub>6</sub>NO (94%) and C<sub>8</sub>H<sub>8</sub>N<sub>2</sub> (6%). The latter component probably is due to the expulsion of a CO molecule from the (M-16)<sup>+</sup> ion. Scheme 7 was substantiated by a comparison of the mass spectrum of 3-amino-1-hydroxy-2(1H)-quinolone with that of the trideuterated derivative, 3-amino-d<sub>2</sub>-1-hydroxy-d-2(1H)-quinolone (144). The ions m/e 176, 159, 132, 131, 104 and 77 in the mass spectrum of the former compound were

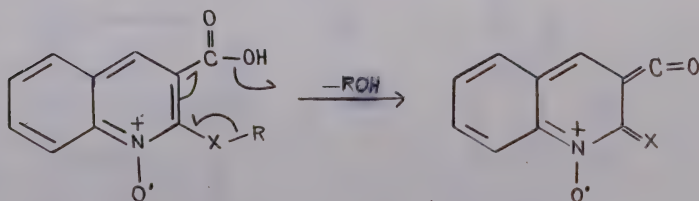




(144)

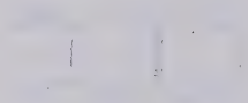
found at 179, 161, 133, 133, 105, 78 and 77 respectively in the deuterated compound. An accurate mass determination of ion  $m/e$  133 in the deuterated compound showed that it was a mixture of  $C_8H_5D_2N_2$  (33%) and  $C_8H_5DNO$  (67%). Appropriate metastable ions were present in the spectrum of the deuterated compound to account for the formation of ion  $m/e$  133.

The loss of a water molecule observed in the mass spectra of 1-hydroxy-2(1H)-quinolone-3-carboxylic acid (138b) and 2-aminoquinoline-3-carboxylic acid 1-oxide (143) (see Figures 7 and 8) is the result of an ortho-effect. This loss of a water molecule is not surprising



138b)  $X-R=OH$

143)  $X-R=NH_2$



1. The first part of the document describes the general principles of the system. It discusses the importance of maintaining accurate records and the need for regular inspections. The text emphasizes the role of the operator in ensuring the safe and efficient operation of the equipment.

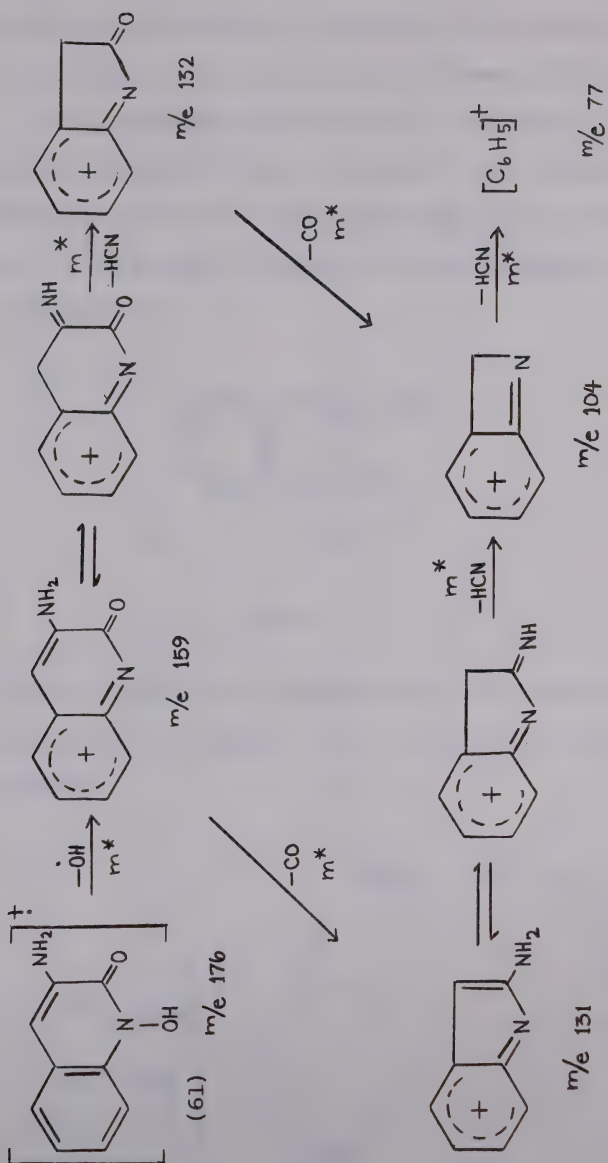
2. The second part of the document provides a detailed description of the components of the system. It includes a list of parts and their functions, as well as a diagram showing the overall layout of the system. The text also discusses the importance of proper maintenance and the need for regular cleaning and lubrication.

3. The third part of the document describes the operating procedures for the system. It includes a list of steps to be followed when starting and stopping the system, as well as a discussion of the safety precautions that must be taken. The text also discusses the importance of proper training and the need for regular safety training for all operators.

4. The fourth part of the document describes the maintenance procedures for the system. It includes a list of tasks to be performed on a regular basis, as well as a discussion of the tools and equipment that are needed. The text also discusses the importance of proper record-keeping and the need for regular maintenance records.



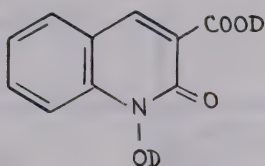
1. The first part of the document describes the general principles of the system. It discusses the importance of maintaining accurate records and the need for regular inspections. The text emphasizes the role of the operator in ensuring the safe and efficient operation of the equipment.



Scheme 7

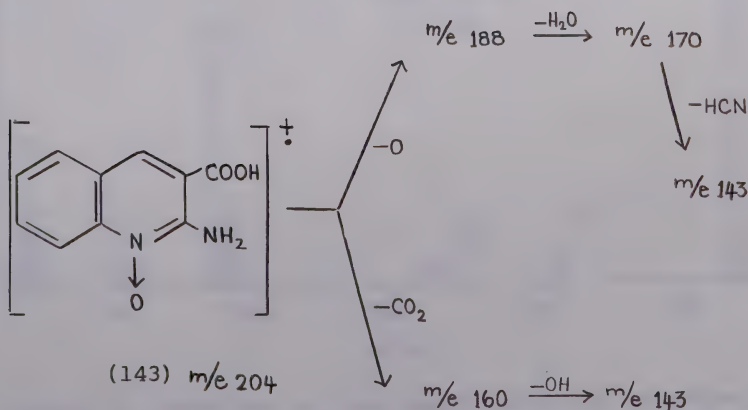


since anthranilic acid and salicylic acid are known to behave similarly on electron impact (Emery, 1960; Biemann, 1962). The fragmentation pathway of 1-hydroxy-2(1H)-quinolone-3-carboxylic acid (Figure 7) was substantiated by interpretation of the mass spectrum of the deuterated compound, 1-hydroxy-d-2(1H)-quinolone-3-carboxylic acid-d (145) (Figure 7).



(145)

Figure 8 shows the fragmentation of 2-aminoquinoline-3-carboxylic acid 1-oxide. The ion  $m/e$  143 could arise in two ways:







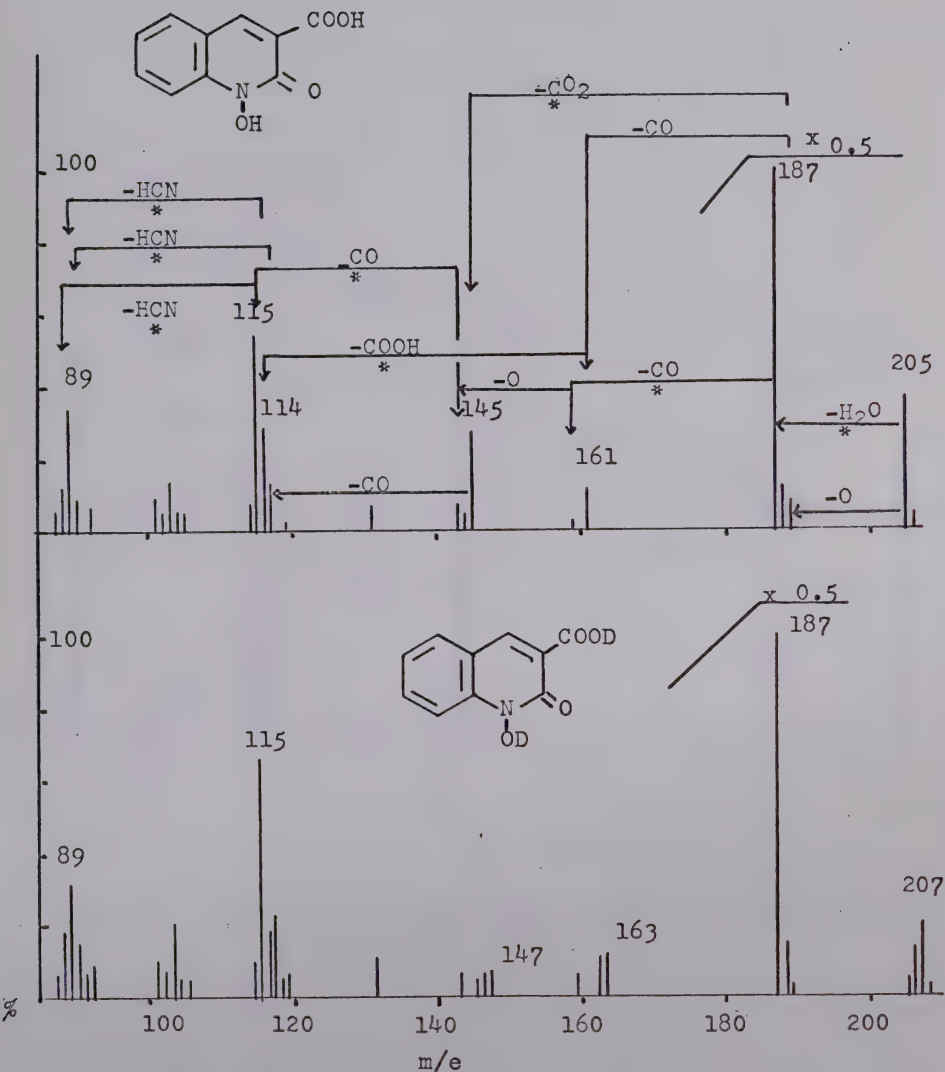


Figure 7: Portions of the mass spectra of 1-hydroxy-2(1H)-quinolone-3-carboxylic acid and 1-hydroxy-d-2(1H)-quinolone-3-carboxylic acid-d.



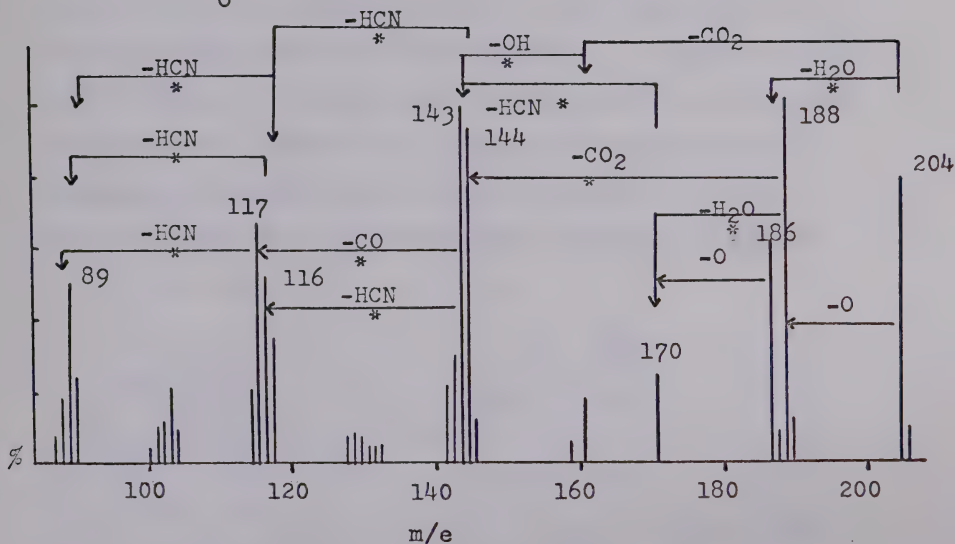
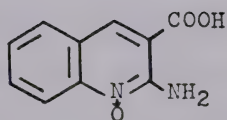
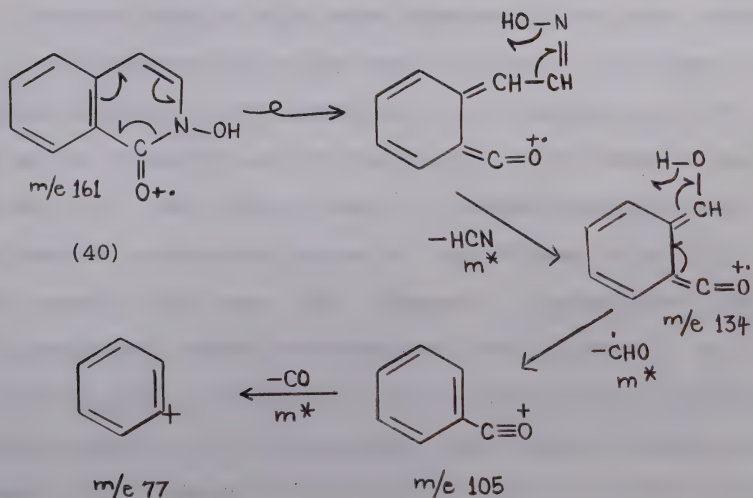


Figure 8: Portion of the mass spectrum of 2-aminoquinoline-3-carboxylic acid 1-oxide.



The peak at 143 was, as expected, a doublet. Accurate mass measurements of the ions  $m/e$  115, 116, 117, 143, 144 and 160 substantiated the proposed fragmentation pathway for this compound.

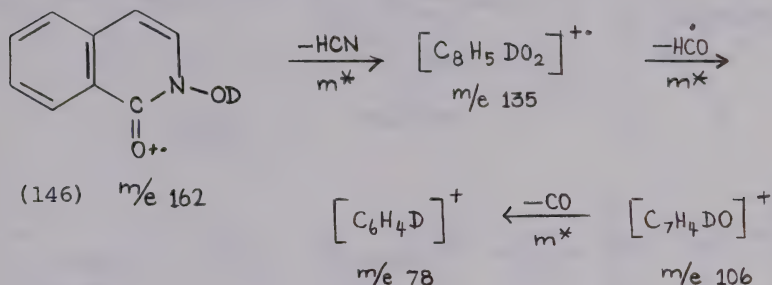
The ions of greatest abundance in the mass spectrum of 2-hydroxy-1(2H)-isoquinolone (40), as seen in Figure 3, are  $m/e$  161, 145, 134, 118, 116, 105, 90 and 89. All of these ions except  $m/e$  134 and 105 can be accounted for by a fragmentation pathway similar to that described for 1-hydroxy-2(1H)-quinolone (21) (Scheme 4). Ions of  $m/e$  134 and 105 can be accounted for by considering the following rearrangement (Scheme 8). Accurate mass determinations supported the identity of ions  $m/e$  134 and 105. In addition, the deuterated compound, 2-hydroxy-d-1(2H)-



Scheme 8



isoquinolone (146) fragmented in a similar manner to give ions  $m/e$  135, 106 and 78. The  $(M-16)^+$  ion in the mass

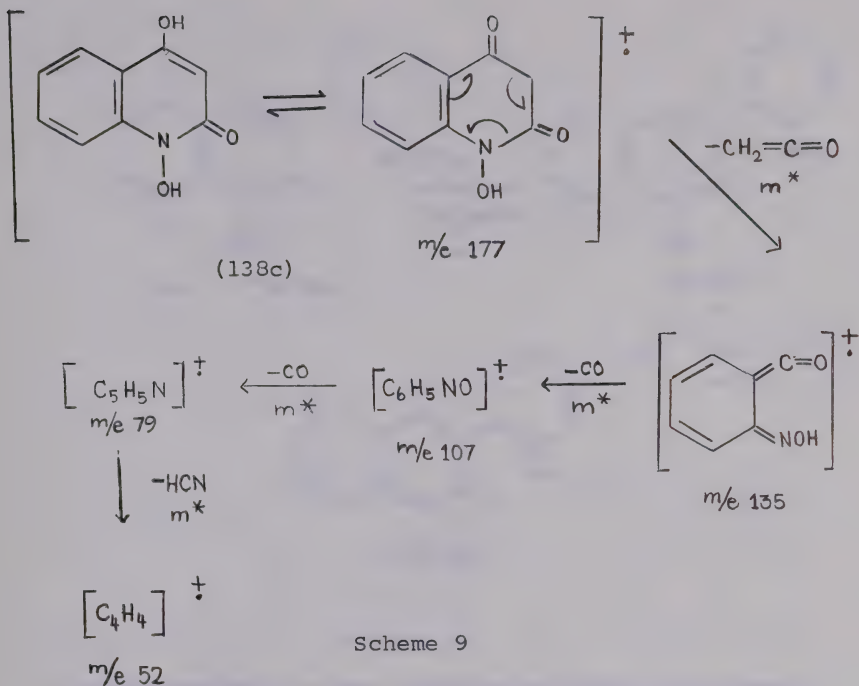


spectrum of 2-hydroxy-1(2H)-isoquinolone (Figure 3) was observed to be the base peak. It was noticed, however, that small changes in source temperature had a profound effect on the relative intensities of the  $(M)^+$  and  $(M-16)^+$  ions in the spectrum of this compound.

Certain similarities were observed in the mass spectra of 1,4-dihydroxy-2(1H)-quinolone (138c) and 4,5-dihydro-5-hydroxy-3-methyl-4-oxoisoxazolo-(4,5-c)-quinoline (139). The major fragment ions of the former (138c) were of  $m/e$  135 and 79. The initial loss of a ketene molecular ion followed by successive losses of two 28 mass units (CO) will account for these ions (Scheme 9). Metastable ions of appropriate masses substantiated this proposal. Although the mass spectrum of 4,5-dihydro-5-hydroxy-3-methyl-4-oxoisoxazolo-(4,5-c)-quinoline (139) was not studied in detail, the presence of a metastable ion at  $m/e$  84.4 suggested that the initial fragmentation was the direct loss



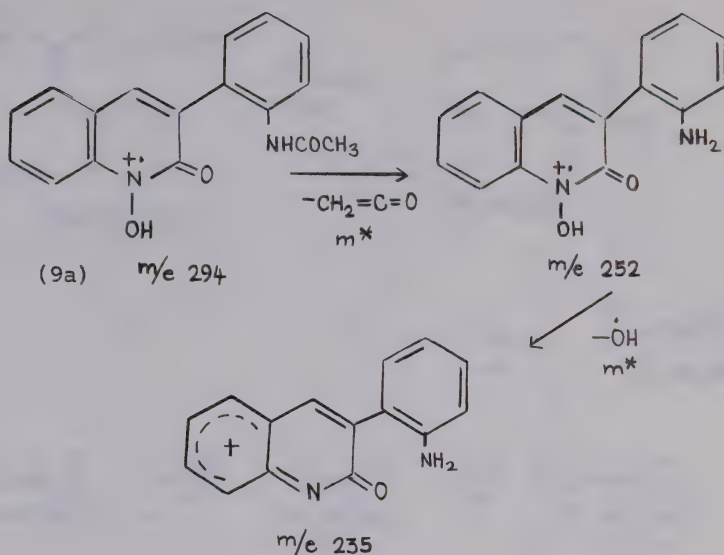




of a  $C_4H_3NO$  fragment from the molecular ion and other appropriate metastable ions indicated that the resulting ion  $m/e$  135 fragmented further similar to that described for 1,4-dihydroxy-2(1H)-quinolone (Scheme 9).

The spectrum of 3-(o-acetamidophenyl)-1-hydroxy-2(1H)-quinolone (9a) was complex. However, the base peak in the spectrum was found to be the ion  $m/e$  235 which was shown, by accurate mass determination, to have the elemental composition  $C_{15}H_{11}N_2O$ . The following Scheme (10) depicts the formation of this ion.

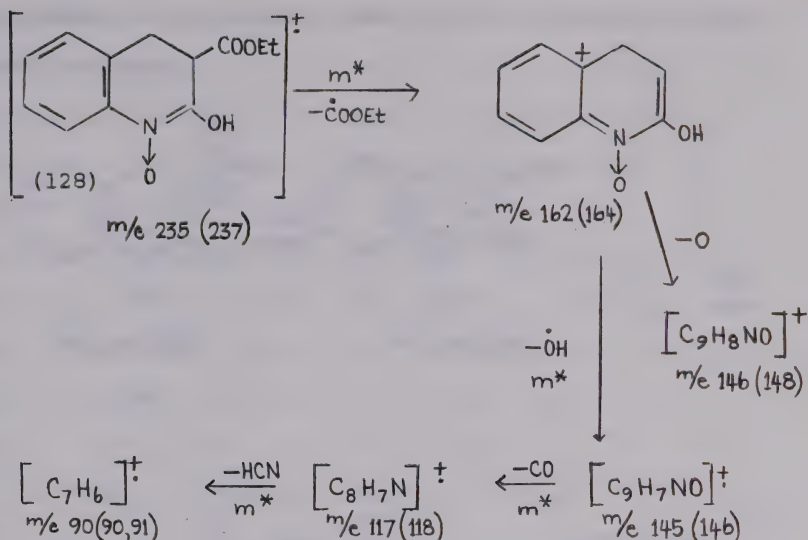




Scheme 10

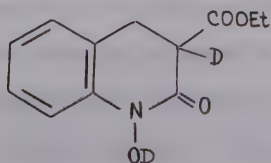
The base peak in the spectrum of ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate (128) was due to the ion  $m/e$  162. The presence of an appropriate metastable ion accounts for the formation of this ion by the expulsion of an ethoxycarbonyl group from the molecular ion. Subsequent losses of an O atom and an OH radical from the ion  $m/e$  162 followed by the loss of 28 (CO) and 27 (HCN) mass units is observed (Scheme 11). The mass spectrum revealed another fragmentation pathway (Scheme 12) which involves the initial loss of an EtOH group followed by a  $C_3O_2$  molecule. Accurate mass determinations of ions  $m/e$  189 and 121 confirmed their identities as  $C_{10}H_7NO_3$  and  $C_7H_7NO$  respectively. The loss





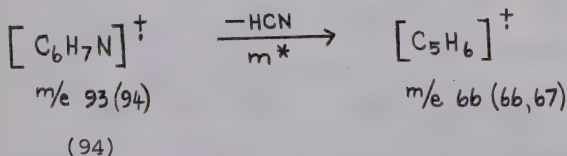
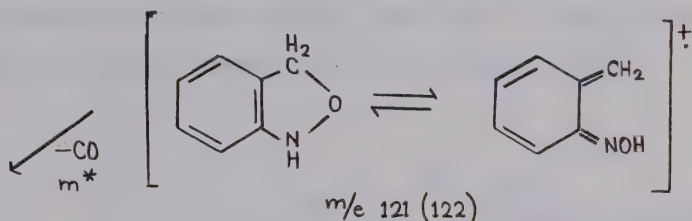
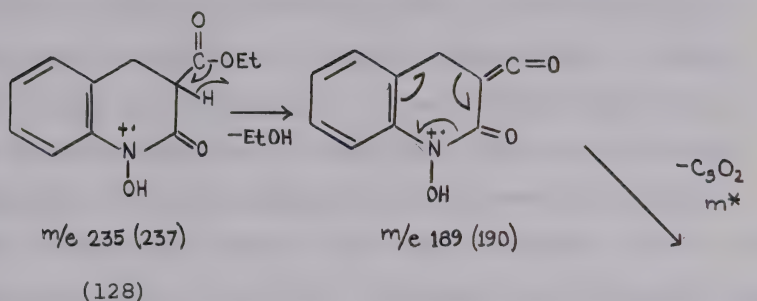
Scheme 11

of the  $\text{C}_3\text{O}_2$  molecule is supported by the presence of a strong metastable ion at  $m/e$  77.5 in the spectrum of the non-labeled compound and by the presence of a metastable ion at  $m/e$  78.3 in the dideuterated compound, ethyl 3,4-dihydro-3-d-1-hydroxy-d-2(1H)-quinolone-3-carboxylate (147). The ions present in the spectrum of the dideuterated compound (147) are given in parenthesis

(14<sup>-</sup>)



in Schemes 11 and 12 and thus aid in confirming the proposed pathways.



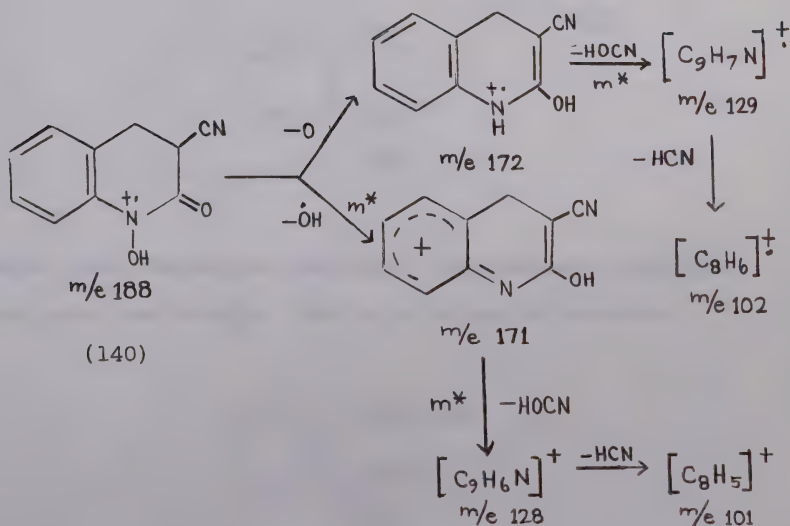
Scheme 12

Although 3-cyano-3,4-dihydro-1-hydroxy-2(1H)-quinolone (140) is structurally related to ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate (128), the mass spectrum differed significantly. The only similarity was an abundant ion at m/e 93 which was shown by accurate mass determination to be  $\text{C}_6\text{H}_7\text{N}$ . The molecular ion





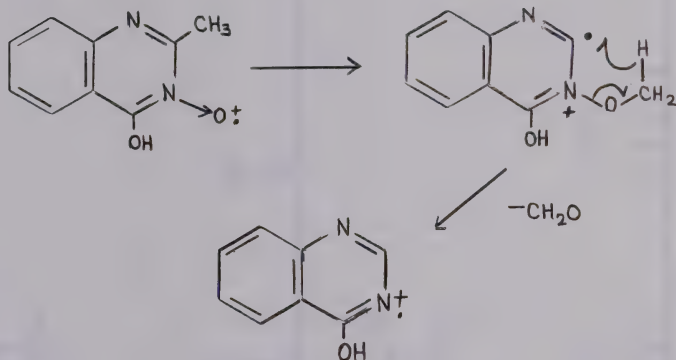
of 3-cyano-3,4-dihydro-1-hydroxy-2(1H)-quinolone was observed as the base peak. The molecular ion decomposed by losing an O atom, an OH radical and a water molecule to give ions of  $m/e$  172, 171 and 170 respectively. These ions fragmented predictably by the further loss of CO, HCN and HCN molecules from each ion. The loss of these molecules was substantiated by appropriate metastable ions. There were however, two other prominent ions in the spectrum at  $m/e$  129 and 128. Accurate mass determinations confirmed that these ions were the result of the loss of an HOCN molecule from the  $(M-16)^+$  and  $(M-17)^+$  ions (Scheme 13). This is another good example of an ortho-effect.



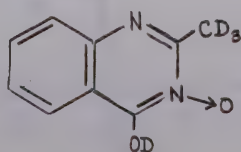
Scheme 13



Labelling studies proved to be invaluable in the study of the 4-hydroxy-2-methylquinazoline-3-oxide (141). This hydroxamic acid behaved uniquely. Although the expulsion of an oxygen atom and an OH radical from the molecular ion did occur, the major initial fragmentation was the loss of 30 mass units (Figure 9). Initially it was thought that a rearrangement such as depicted by the following was responsible for this loss:



However, the deuterated compound, 4-hydroxy-d<sub>3</sub>-2-methyl-d<sub>3</sub>-quinazoline-3-oxide (148) also shows an abundant



(148)



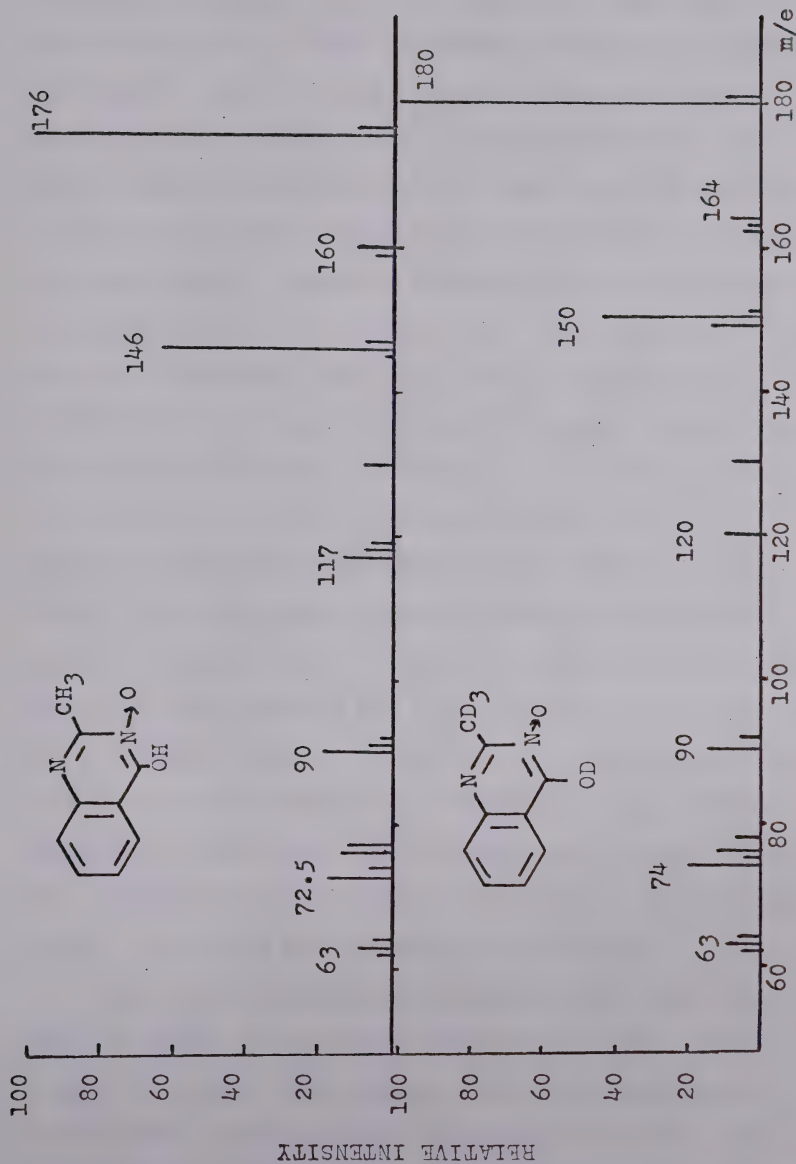


Figure 9: Portions of the mass spectra of 4-hydroxy-2-methylquinazoline-3-oxide and 4-hydroxy-d<sub>3</sub>-2-methyl-d<sub>3</sub>-quinazoline-3-oxide.

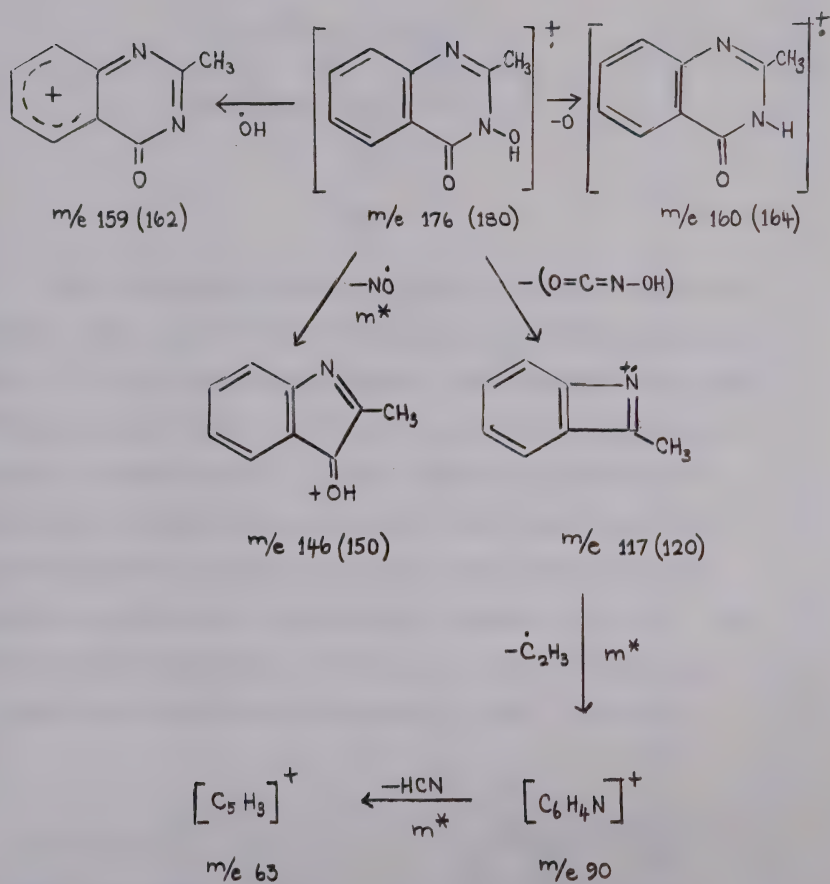


$(M-30)^+$  ion and not an  $(M-32)^+$  ion. An accurate mass determination showed that the elemental composition of the ion  $m/e$  146 in 4-hydroxy-2-methylquinazoline-3-oxide was  $C_9H_8NO$ . This is due to the expulsion of nitric oxide from the molecular ion. The expulsion of nitric oxide from the molecular ion has been observed recently in the mass spectrum of N-hydroxyphthalimide (Bowie, Hearn and Ward, 1969). Scheme 14 accounts for the major ions in the spectrum of the quinazoline. Ions observed in the deuterated compound (148) are given in parenthesis. Ions of mass 117 and 90 were confirmed by accurate mass determinations but how they are formed is not known. A metastable ion at  $m/e$  69.2 in the spectrum of the non-deuterated compound (141) supports the loss of a  $C_2H_3$  radical (or simultaneous loss of  $CH=CH$  molecule and an H radical) from the  $m/e$  117 ion but no confirmatory metastable ion was observed for a  $m/e$  120  $\rightarrow$   $m/e$  90 transition of the labeled compound. Cleavage of a  $C_2H_3$  radical is known to occur in compounds possessing a vinylic bond (Meyerson and McCollum, 1963; Budzikiewicz et al, 1964). This suggests that the formation of the ion  $m/e$  90 from  $m/e$  117 involves a rearrangement of the latter.

Because of interesting fragmentations obtained on electron impact of quinoline hydroxamic acids, it was decided to extend this present study to other classes of hydroxamic acids having the general structure (149) in which  $X=O, S, SO_2$ . This study has revealed that the



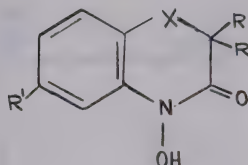




Scheme 14



three classes, benzoxazines, benzothiazines and benzothiazine 1,1-dioxides fragment initially in different ways.



(149)

The hydroxamic acids examined in the benzoxazine series were: 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (77); 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzoxazine (150a); 6-chloro-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (150b); 2-ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (131) and 2-n-butyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (150c). The mass spectra are included in Figures 10 to 12. The benzoxazine hydroxamic acids behave quite differently to the simple quinoline hydroxamic acids. They are

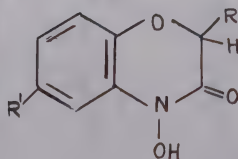
77)  $R=R'=H$

150a)  $R=R'=Me$

150b)  $R=H, R'=Cl$

131)  $R=Et, R'=H$

150c)  $R=nBu, R'=H$



(150)



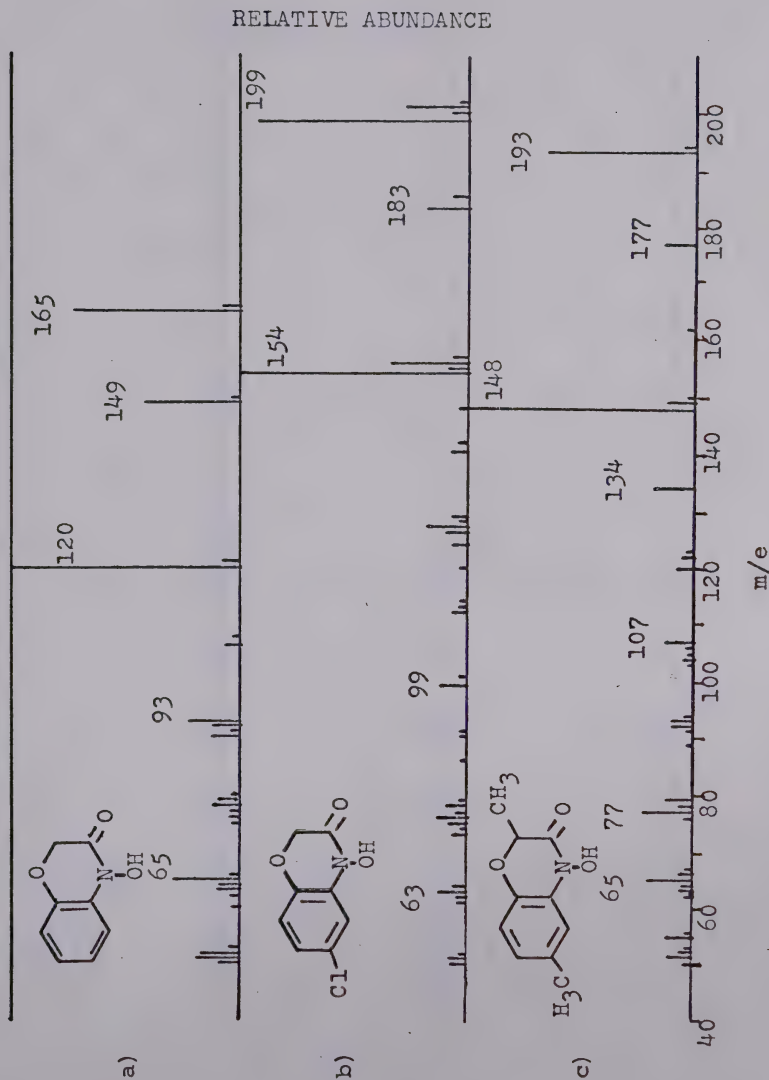


Figure 10: Mass spectra of a) 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine, b) 6-chloro-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine and c) 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzoxazine.



## RELATIVE ABUNDANCE

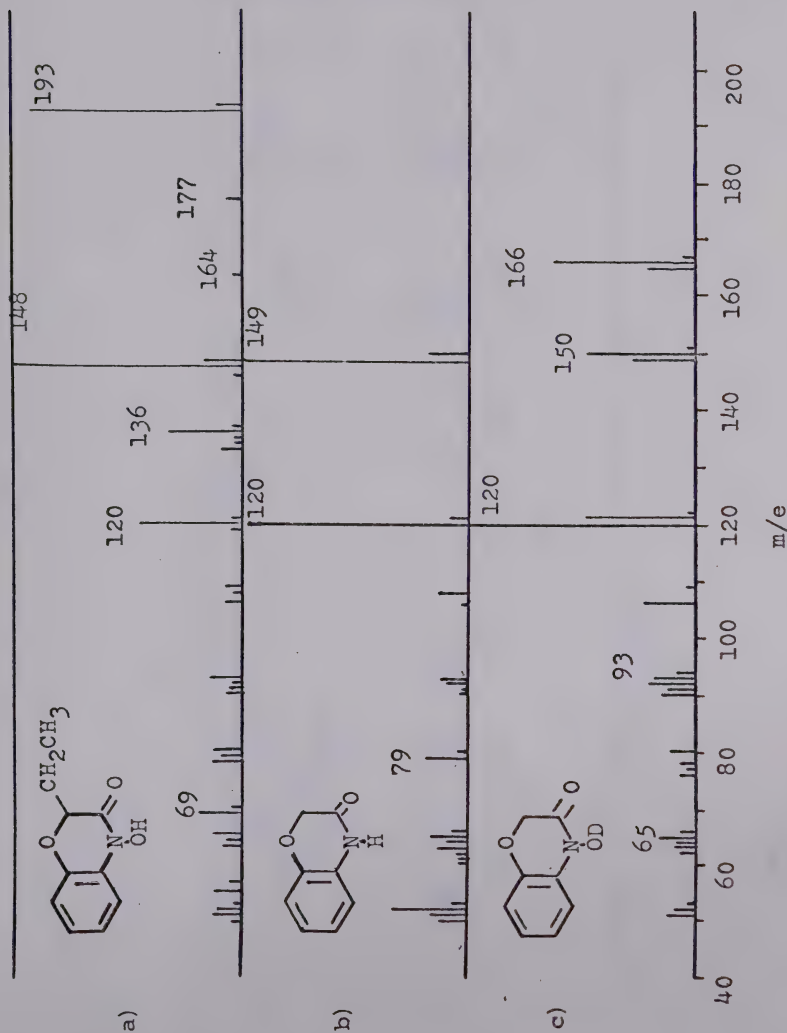


Figure 11: Mass spectra of a) 2-ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine, b) 3,4-dihydro-3-oxo-2H-1,4-benzoxazine and c) 3,4-dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzoxazine.





RELATIVE ABUNDANCE

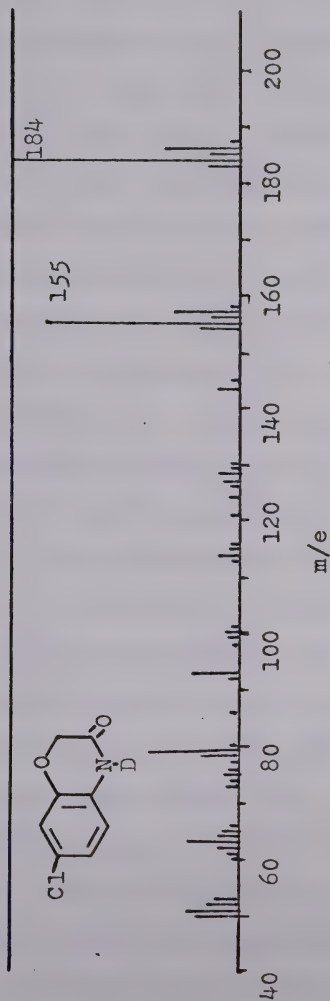


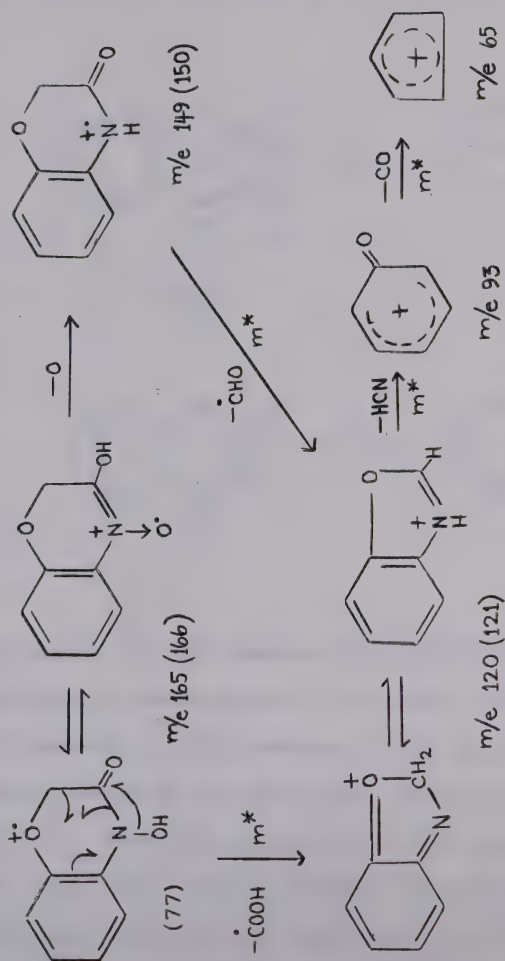
Figure 12: Mass spectrum of 7-chloro-3,4-dihydro-4-d-3-oxo-2H-1,4-benzoxazine.



characterized by the presence of a strong  $(M-45)^+$  ion, the result of the loss of a COOH radical from the molecular ion. In fact, this ion was the base peak in all the benzoxazine hydroxamic acids studied. The presence in each spectrum of a metastable ion of appropriate mass indicated that the  $M^+ \longrightarrow (M-45)^+$  transition was a one-step elimination. Other noticeable features of this class of hydroxamic acids were the absence of  $(M-17)^+$  ions, and the presence of  $(M-16)^+$  ions of relatively low abundance. The parent hydroxamic acid, 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (77) decomposed in the manner shown by Scheme 15. Accurate mass determinations confirmed the elemental composition of ions  $m/e$  120 and 93 to be  $C_7H_6NO$  and  $C_6H_5O$  respectively. The pathway shown by Scheme 15 was substantiated by examining the mass spectrum of the deuterated compound 3,4-dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzoxazine (151). This latter compound fragmented similarly and the ions observed are given in parenthesis in Scheme 15. The lactam, 3,4-dihydro-3-oxo-2H-1,4-benzoxazine (78), fragmented as expected, in the same way as the  $(M-16)^+$  ion of the parent hydroxamic acid. 6-Chloro-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (150b) also decomposed in the same manner as 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine.

The origin of the hydrogen atom contained in the CHO radical which is expelled from the  $(M-16)^+$  ions of the two hydroxamic acids was investigated. Two pathways are

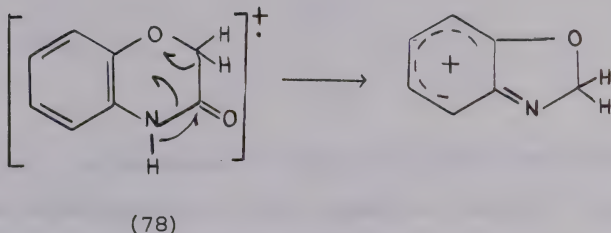




Scheme 15



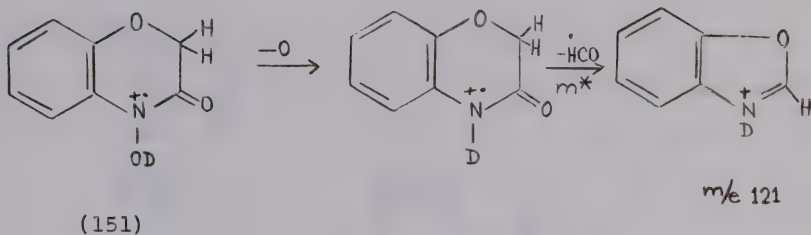
possible. The hydrogen could originate from the methylene group or it could be the atom present originally in the  $\text{-CO-N(OH)-}$  grouping of the molecule:



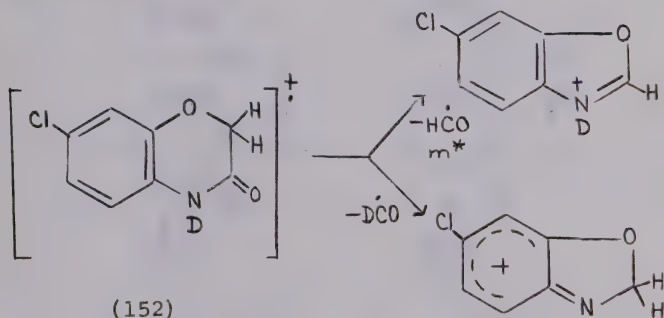
Examination of the spectra of 3,4-dihydro-4-hydroxy-d-3-oxo-2 $\underline{\text{H}}$ -1,4-benzoxazine (151) and 7-chloro-3,4-dihydro-4-d-3-oxo-2 $\underline{\text{H}}$ -1,4-benzoxazine (152) proved that the hydrogen atom of the methylene group is at least partly involved. The mass spectrum of the deuterated hydroxamic acid (151) had an abundant ion of  $m/e$  121 (47%) which was absent in the spectra of 3,4-dihydro-4-hydroxy-3-oxo-2 $\underline{\text{H}}$ -1,4-benzoxazine (77) and the 6-chloro-derivative (150b) and must therefore have arisen as follows:





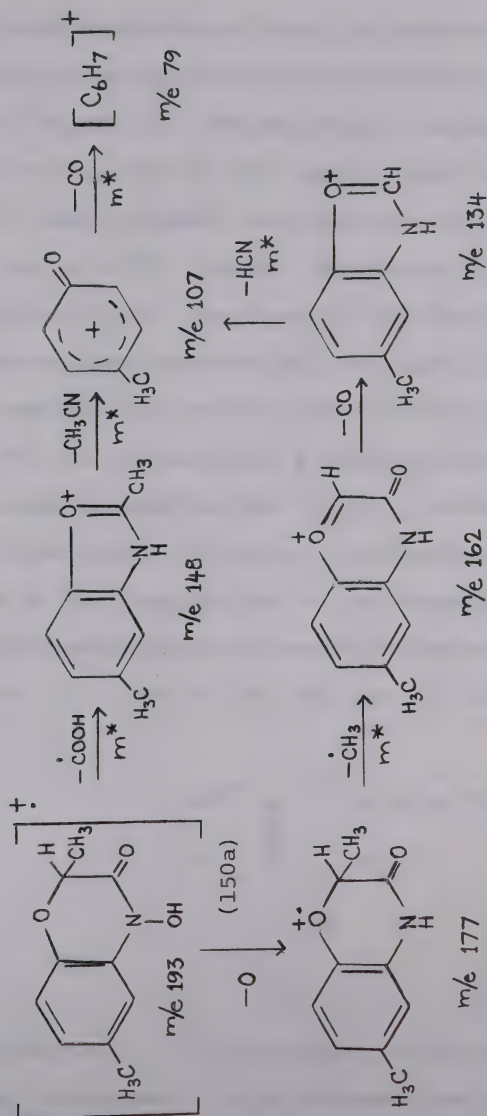


Similarly, the spectrum of 7-chloro-3,4-dihydro-4-d-3-oxo-2H-1,4-benzoxazine (152) showed an  $(M-HCO)^+$  ion of 85% relative abundance and an  $(M-DCO)^+$  ion of only 15% abundance.



Major ions of mass  $m/e$  193, 177, 148, 134 and 107 were present in the mass spectrum of 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzoxazine (150a). The ions of  $m/e$  177 and 148 are typical of the benzoxazine series, i.e.  $(M-16)^+$  and  $(M-45)^+$  respectively. The other ions,  $m/e$  134 and 107, can be explained by further decomposition of the  $(M-16)^+$  and  $(M-45)^+$  ions (Scheme 16).

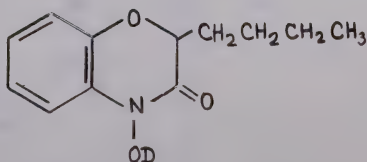




Scheme 16



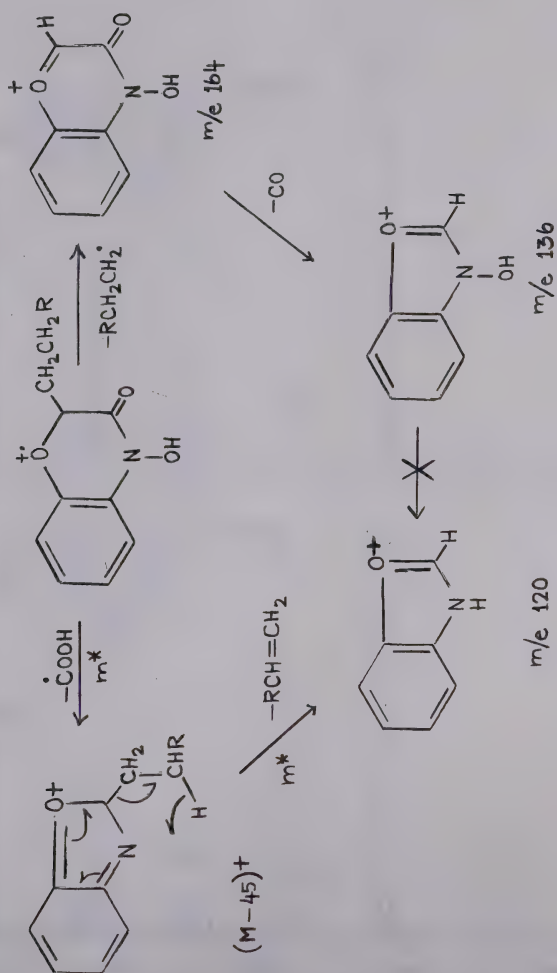
The two remaining benzoxazine hydroxamic acids, 2-ethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (131) and 2-n-butyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (150c) (Figure 13), decomposed by the initial characteristic loss of the COOH radical and then a McLafferty type of rearrangement gave the ion m/e 120 (Scheme 17). This ion, m/e 120, further decomposed in the same way as the ion of the same mass in the spectrum of 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine (Scheme 15). Both spectra (131 and 150c) exhibited ions of m/e 164 and 136, the latter being a prominent ion and having the elemental composition  $C_7H_6NO_2$ . Scheme 17 shows how these ions could originate. This pathway is substantiated by the examination of the spectrum of 2-n-butyl-3,4-dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzoxazine (153) (Figure 13). Ions of m/e 165 and 137 are observed in



(153)

this spectrum. It is not known how the ion m/e 136 further decomposes. It is evident that it does not lose an oxygen atom to give the ion m/e 120 because the labeled compound did not have a corresponding ion,





Scheme 17





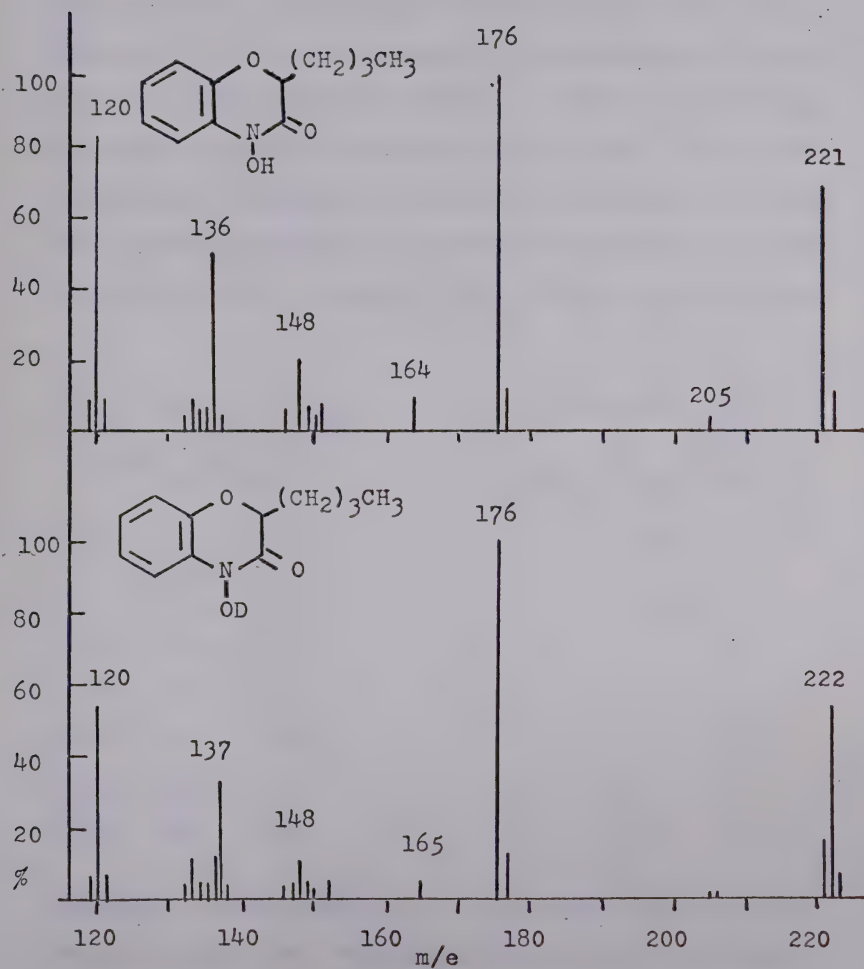
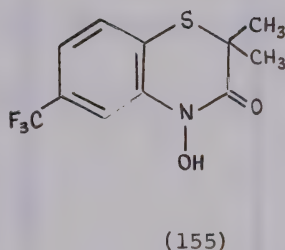
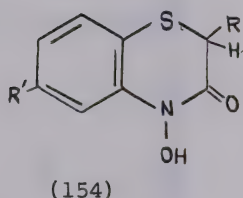


Figure 13: Portions of the mass spectra of 2-n-butyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine and 2-n-butyl-3,4-dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzoxazine.



m/e 121.

A group of 2H-1,4-benzothiazines were the next hydroxamic acids to be investigated. The compounds studied included: 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine (3); 6-trifluoromethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine (154a); 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine (154b), 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzothiazine (154c) and 6-trifluoromethyl-3,4-dihydro-4-hydroxy-2,2-dimethyl-3-oxo-2H-1,4-benzothiazine (155). Two related lactams



	R	R'
3)	H	H
154a)	H	CF <sub>3</sub>
154b)	Me	H
154c)	Me	Me

were also investigated, i.e. 3,4-dihydro-3-oxo-2H-1,4-benzothiazine (156) and 6-trifluoromethyl-3,4-dihydro-2,2-dimethyl-3-oxo-2H-1,4-benzothiazine (157). The mass spectra of most of these compounds are included in Figures 14 and 15. Electron impact on this series of



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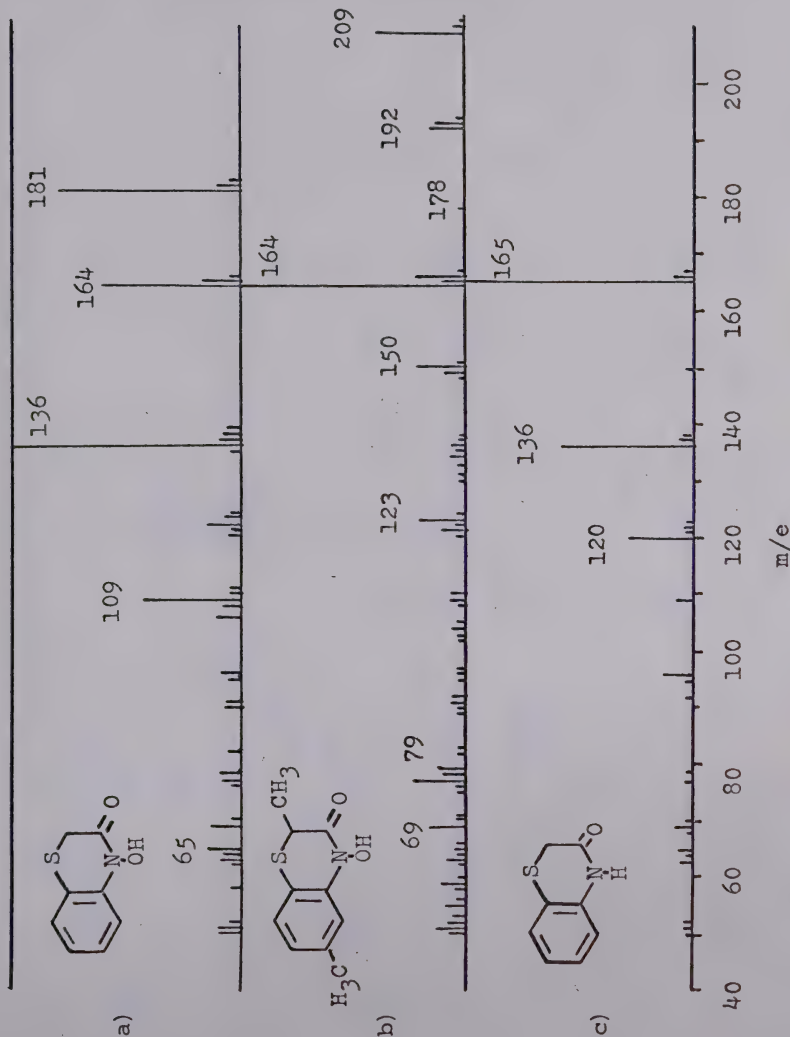


Figure 14: Mass spectra of a) 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine, b) 3,4-dihydro-4-hydroxy-2,6-dimethyl-1,4-benzothiazine and c) 3,4-dihydro-3-oxo-2H-1,4-benzothiazine.



RELATIVE ABUNDANCE

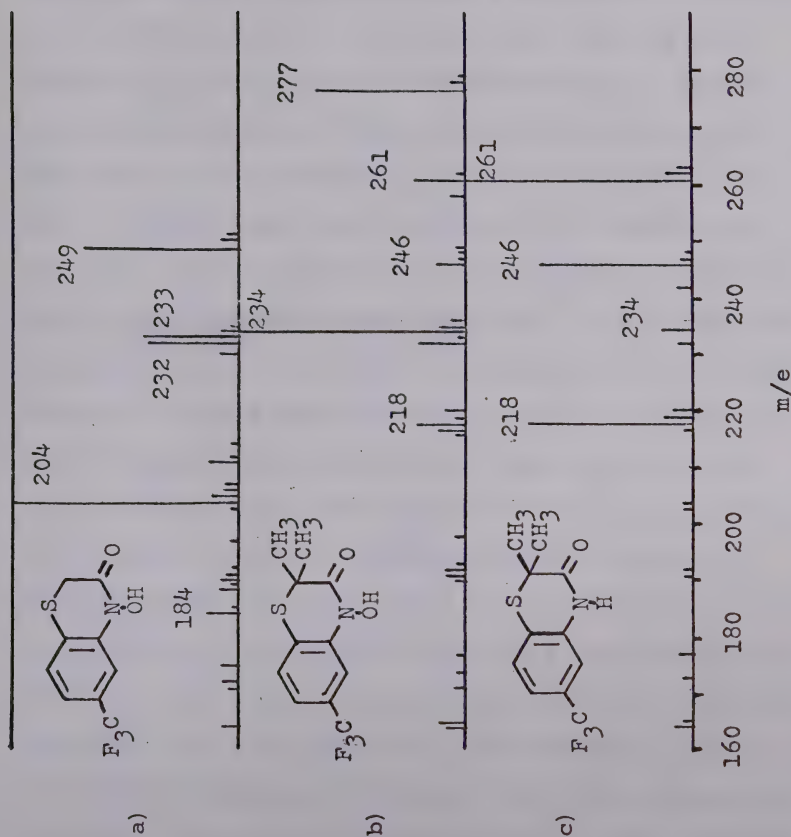
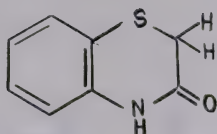


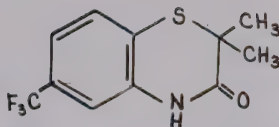
Figure 15: Mass spectra of a) 6-trifluoromethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine, b) 6-trifluoromethyl-3,4-dihydro-2,2-dimethyl-3-oxo-2H-1,4-benzothiazine and c) 6-trifluoromethyl-3,4-dihydro-2,2-dimethyl-3-oxo-2H-1,4-benzothiazine.







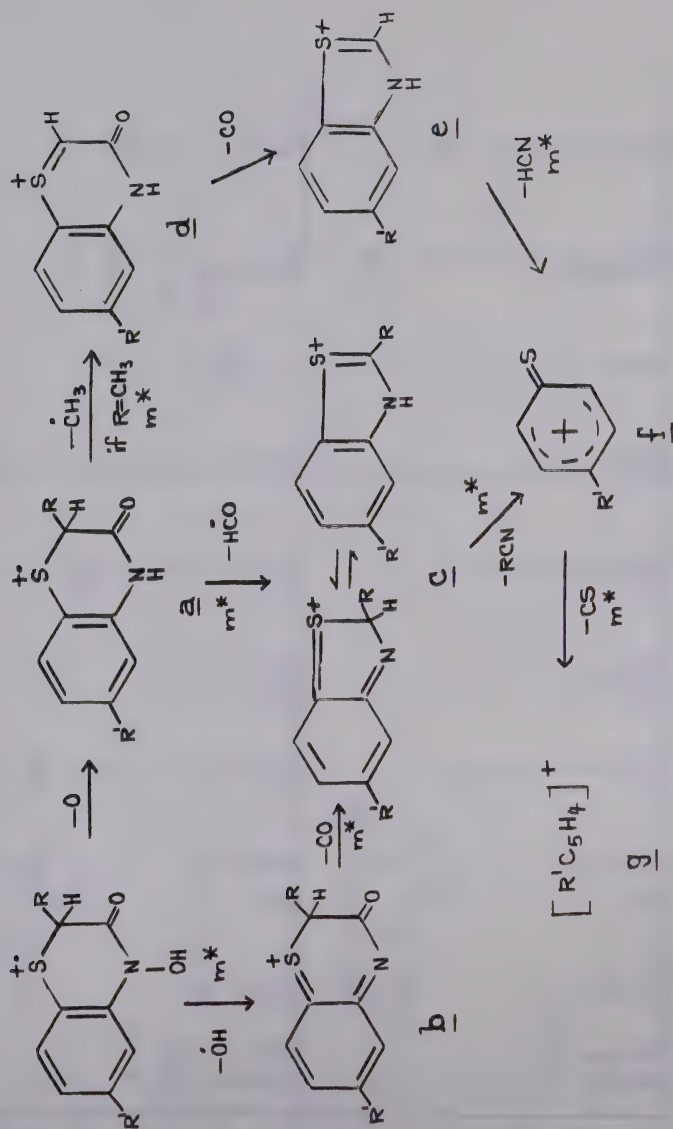
(156)



(157)

hydroxamic acids resulted in the formation of an  $(M-45)^+$  ion in each spectrum. An ion of this mass was also observed in the spectra of the benzoxazines. However, none of the spectra of the benzothiazines contained a metastable ion to support a direct  $M^+ \rightarrow (M-45)^+$  transition. Instead there were metastable ions present for the loss of an OH radical and a CO molecule or for the loss of an HCO radical from the  $(M-16)^+$  ion. Subsequent decompositions of the  $(M-45)^+$  ion were similar to those observed with the benzoxazines (Scheme 4). The postulated fragmentations in Scheme 18 were substantiated by i) accurate mass determination of ions c, e, f and g in the spectrum of 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine (154b), ii) by examination of the spectrum of 3,4-dihydro-3-oxo-2H-1,4-benzothiazine (156) in which ions c, f and g were present and iii) by examination of the spectrum of the deuterated compound, 3,4-dihydro-4-hydroxy-d-2-methyl-3-oxo-2H-1,4-benzothiazine (158) (Figure 16) in which ions a, d and e carried the deuterium label.





Scheme 18



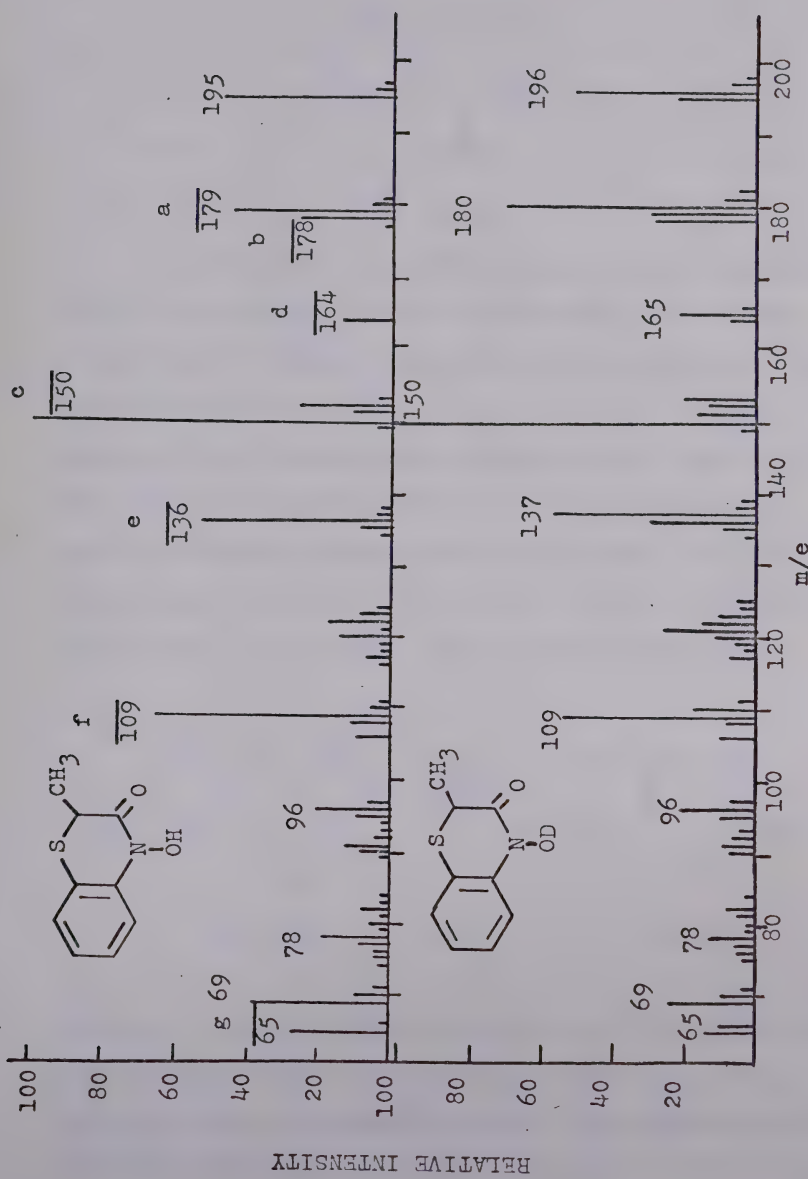
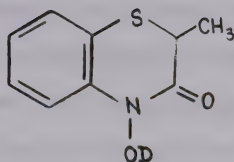


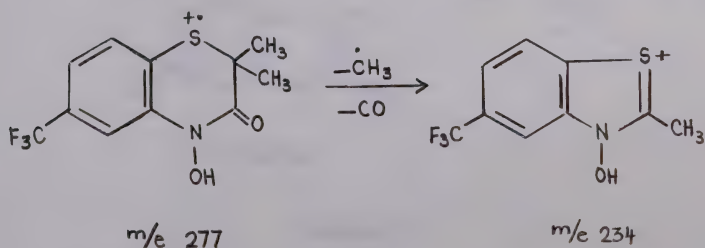
Figure 16: Portions of the mass spectra of 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine and 3,4-dihydro-4-hydroxy-d-2-methyl-3-oxo-2H-1,4-benzothiazine.





(158)

In contrast to the other benzothiazine hydroxamic acids, the spectrum of 6-trifluoromethyl-3,4-dihydro-4-hydroxy-2,2-dimethyl-3-oxo-2H-1,4-benzothiazine (155) did not possess an  $(M-17)^+$  ion. An  $(M-45)^+$  ion was observed but the base peak was the  $(M-43)^+$  ion of  $m/e$  234 which was found by accurate mass measurement to have an elemental composition of  $C_9H_7F_3NOS$ . This ion is probably formed by the loss of a  $CH_3$  radical and a CO molecule. An  $(M-43)^+$  ion is also observed as an

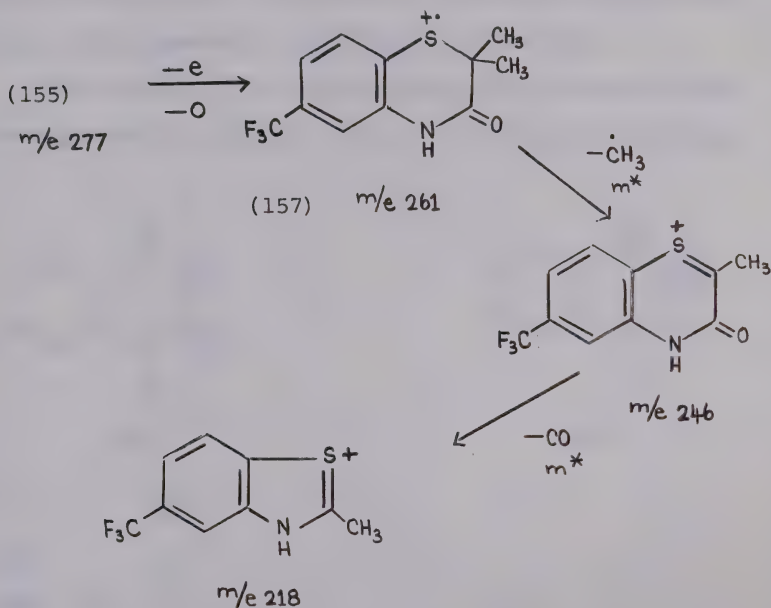


abundant ion in the other benzothiazine hydroxamic acids possessing a methyl group in the 2-position. Other abundant ions in the spectrum of 6-trifluoromethyl-3,4-dihydro-4-hydroxy-2,2-dimethyl-3-oxo-2H-1,4-benzothia-





zine had  $m/e$  261, 246 and 218, all of which were prominent in the corresponding lactam, 6-trifluoromethyl-3,4-dihydro-2,2-dimethyl-3-oxo-2H-1,4-benzothiazine (157). The following Scheme (19) was substantiated by accurate mass determinations and accounts for the three ions just mentioned. The ion  $m/e$  218 decomposed further by the



Scheme 19

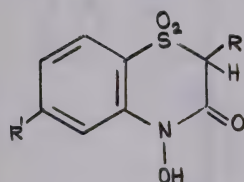
loss of  $CH_3CN$  and  $CS$  molecules which is similar in behaviour to ion c of Scheme 18.

Compounds containing trifluoromethyl groups may be expected to form  $(M-F)^+$ ,  $(M-CF_2)^+$ ,  $(M-CF_3)^+$  and  $CF_3^+$  ions (Budzikiewicz, Djerassi and Williams, 1967c). The

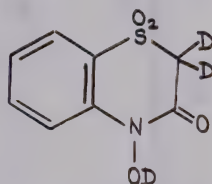


three trifluoromethyl compounds examined in this study (154a, 155, 157) formed a weak (approximately 7% relative abundance) but diagnostic  $(M-F)^+$  ion; however,  $(M-CF_2)^+$  and  $(M-CF_3)^+$  ions were absent. A weak ion,  $m/e$  69, was observed in all spectra, but as the other non-fluorinated benzothiazine hydroxamic acids also gave an ion of this mass, it cannot be considered diagnostic of the trifluoromethyl compounds investigated.

The spectra of 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (159a), 3,4-dihydro-4-hydroxy-



(159)



(160)

	R	R'
159a)	H	H
159b)	Me	H
159c)	H	Me

2-methyl-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (159b) and the 6-methyl derivative, 3,4-dihydro-4-hydroxy-6-methyl-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (159c) were examined (Figures 17 and 18). This group of



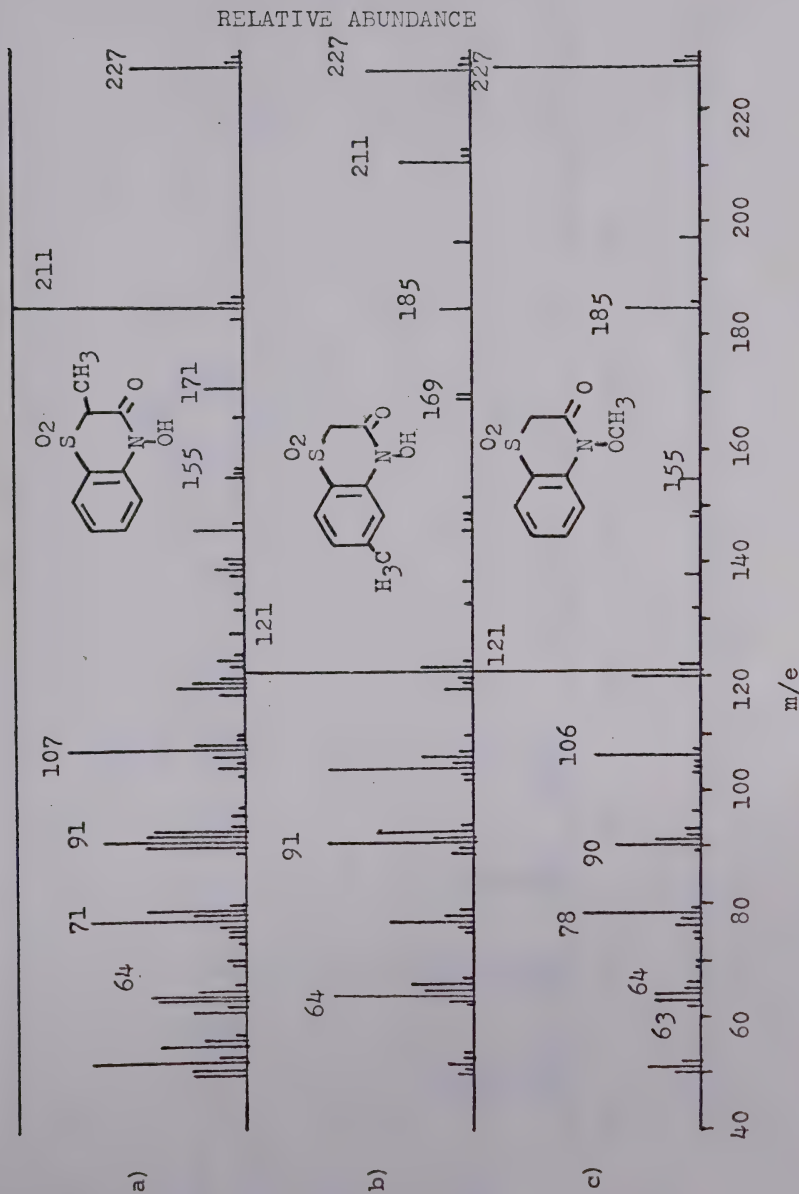


Figure 17: Mass spectra of a) 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine 1,1-dioxide, b) 3,4-dihydro-4-hydroxy-6-methyl-3-oxo-2H-1,4-benzothiazine 1,1-dioxide and c) 3,4-dihydro-4-methoxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide.



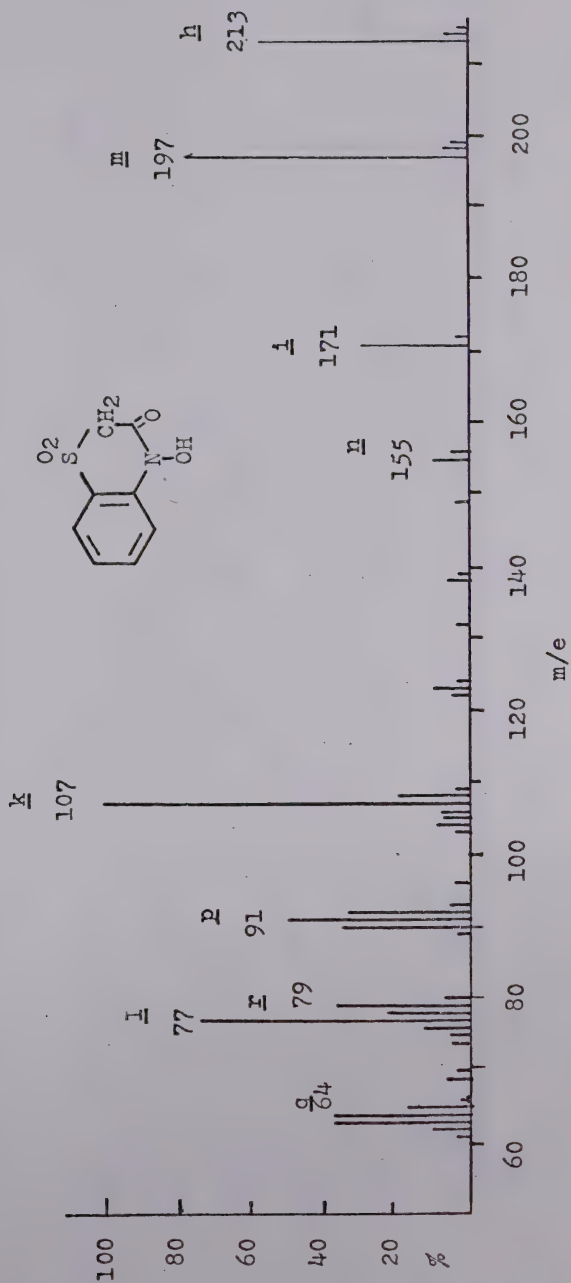


Figure 18: Portion of the mass spectrum of 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide.





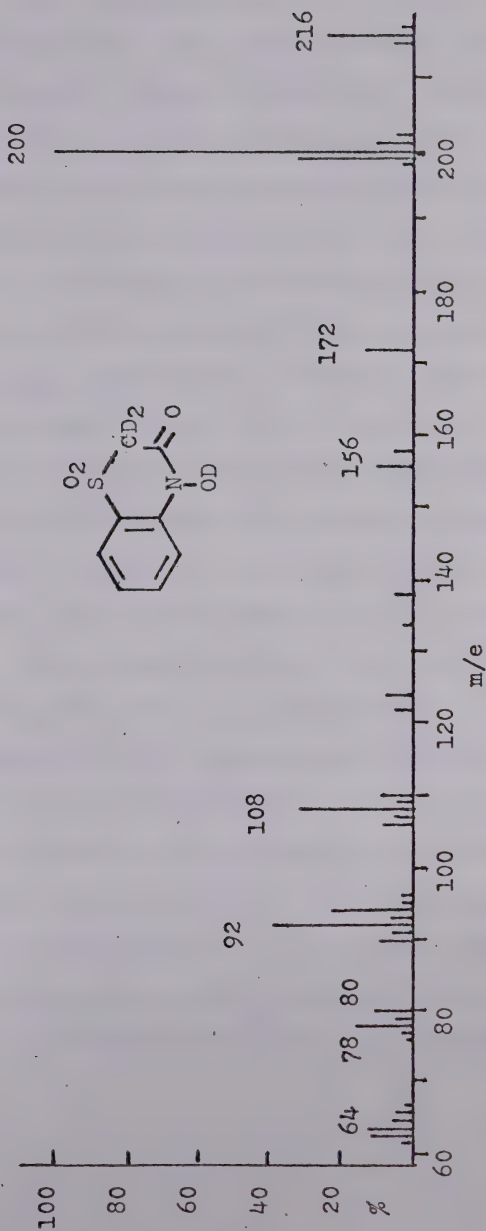


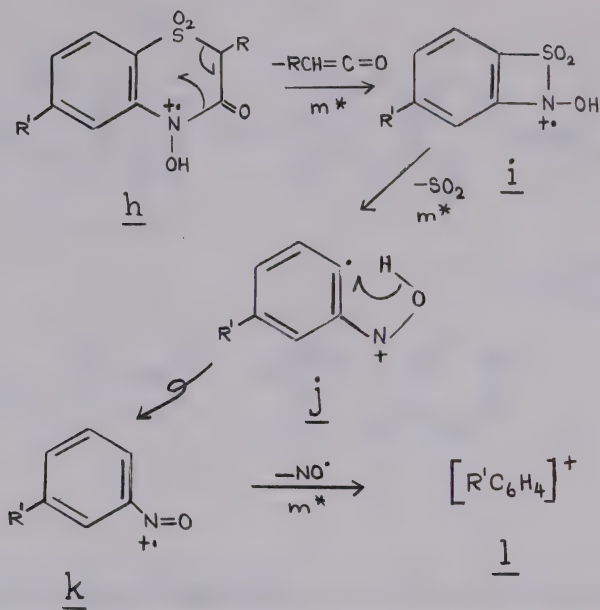
Figure 19: Portion of the mass spectrum of 3,4-dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzothiazine-2-d<sub>2</sub> 1,1-dioxide.



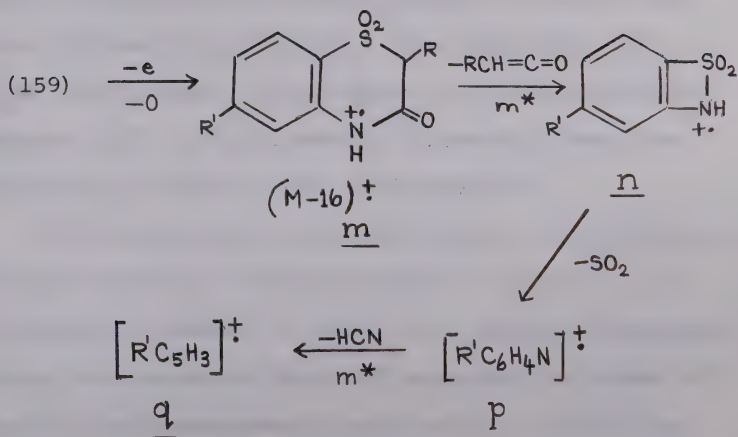
hydroxamic acids decomposed to give abundant  $(M-16)^+$  and  $(M-RHC=C=O)^+$  ions, but neither  $(M-17)^+$  nor  $(M-45)^+$  ions were formed. Scheme 20 shows how these three hydroxamic acids primarily fragmented. Ions k and l were abundant ions in each spectrum and an accurate mass measurement of the ions i, k and l in the mass spectrum of 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide confirmed that they were  $C_6H_5NO_3S$ ,  $C_6H_5NO$  and  $C_6H_5$  respectively. Further support for this fragmentation mode (Scheme 20) was obtained by examination of the spectrum of the trideuterated compound, 3,4-dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzothiazine-2-d<sub>2</sub> 1,1-dioxide (160) (Figure 19). Another fragmentation pathway for this group of hydroxamic acids is shown in Scheme 21. The decompositions of the  $(M-16)^+$  ion are unexceptional and have been substantiated by examination of the spectrum of the trideuterated compound (160).

The presence of metastable ions of appropriate mass in the spectra of the three non-deuterated benzothiazine 1,1-dioxides (159a, b and c) revealed that ion j (Scheme 20) decomposed in still another way, by the loss of a CO and then an HCN molecule. Such an observation may be explained in terms of an OH migration:



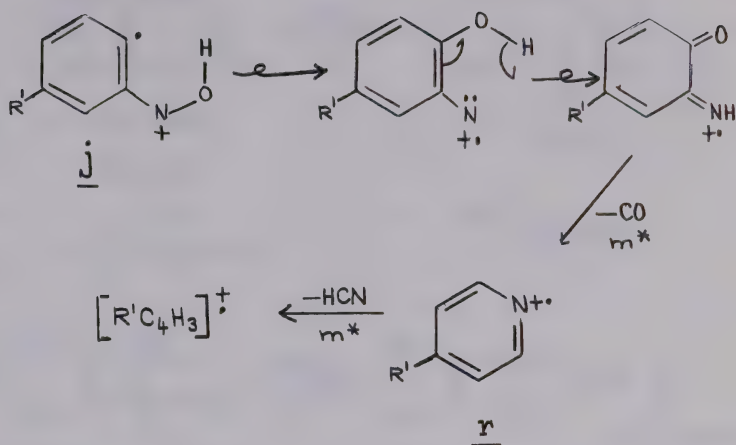


Scheme 20



Scheme 21



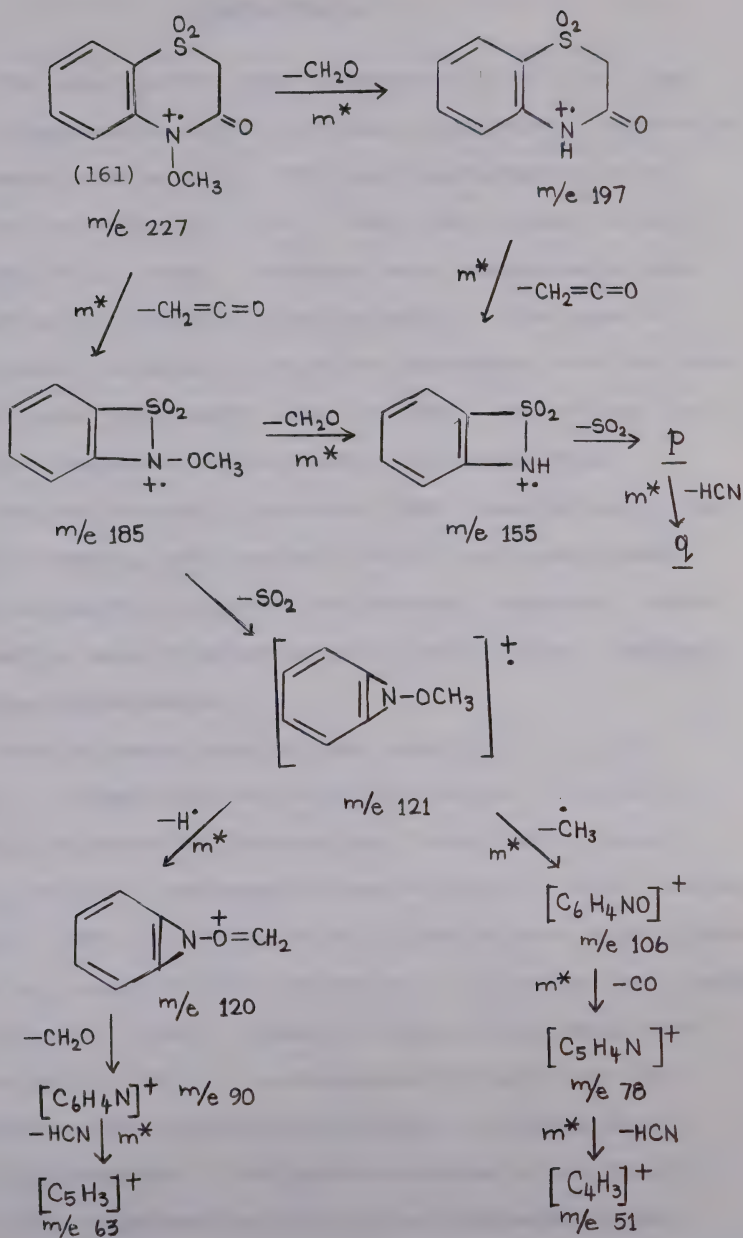


Ion r in the mass spectrum of 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (159a) was found by accurate mass determination to have an elemental composition of  $\text{C}_5\text{H}_5\text{N}$ . The equivalent ion,  $\text{C}_5\text{H}_4\text{DN}$ , was present in the spectrum of the deuterated compound (160). Its elemental composition was also confirmed by accurate mass determination.

The presence of a methoxyl group in 3,4-dihydro-4-methoxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (161) influenced the manner in which this compound decomposed. Appropriate decompositions are suggested in Scheme 22. Accurate mass measurements were made to confirm the compositions of  $m/e$  185, 155, 121, 120, 106, 91 and 90.







Scheme 22



### Experimental

The mass spectra were determined by Dr. A.M. Hogg and his associates in the Chemistry Department, University of Alberta with an A.E.I. MS9 mass spectrometer at an ionizing potential of 70 eV. The direct probe method was used in all cases. Accurate mass measurements were carried out by the peak matching method. The spectra were plotted in terms of relative abundance, with the most intense peak (base peak) taken as 100%. Peaks less than 2% of the abundance of the base peak were ignored.

Nuclear magnetic resonance (NMR) spectra were determined in DMSO-d<sub>6</sub> solution on a Varian A-60 spectrometer using tetramethylsilane as the internal standard. Infra-red spectra were recorded as nujol mulls using a Beckman IR10 Spectrophotometer.

The following samples were provided by Dr. R.T. Coutts: 3-cyano-1-hydroxy-2(1H)-quinolone, 3-cyano-3,4-dihydro-1-hydroxy-2(1H)-quinolone, 1-acetoxy-3-cyano-2(1H)-quinolone, 2-aminoquinoline-3-carboxylic acid 1-oxide (Coutts, 1969); 1,4-dihydroxy-2(1H)-quinolone, 4,5-dihydro-5-hydroxy-3-methyl-4-oxoisoxazolo-(4,5-c)-quinoline (Coutts and Wibberley, 1963); 3-amino-1-hydroxy-2(1H)-quinolone (Coutts et al., 1969); 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine, 6-chloro-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine, 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzoxazine (Coutts and Hindmarsh, 1966); 3,4-dihydro-3-oxo-2H-1,4 benzoxazine; 3,4-dihydro-4-



hydroxy-3-oxo-2H-1,4-benzothiazine, 3,4-dihydro-3-oxo-2H-1,4-benzothiazine, 6-trifluoromethyl-3,4-dihydro-2,2-dimethyl-3-oxo-2H-1,4-benzothiazine (Coutts, Barton and Smith, 1966); 6-trifluoromethyl-3,4-dihydro-4-hydroxy-2,2-dimethyl-3-oxo-2H-1,4-benzothiazine, 6-trifluoromethyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine, 3,4-dihydro-4-hydroxy-2,6-dimethyl-3-oxo-2H-1,4-benzothiazine (Coutts, Peel and Smith, 1965); 3,4-dihydro-4-hydroxy-6-methyl-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (Coutts and Smith, 1967); 3,4-dihydro-4-methoxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide (Coutts et al, 1968). Mr. N.J. Pound provided 3-(o-acetamidophenyl)-1-hydroxy-2(1H)-quinolone (Abramovitch, Coutts and Pound, 1967), 4-hydroxy-2-methylquinazoline-3-oxide (Harrison and Smith, 1960) and 7-chloro-3,4-dihydro-3-oxo-2H-1,4-benzoxazine (Coutts and Pound, 1969). The preparation of the other non-deuterated compounds are reported elsewhere in this thesis. All compounds were purified by dissolving them in 10% sodium carbonate solution and extracting the impurities with ether. The aqueous layer was then acidified and extracted with ether. Evaporation of this ether layer gave the hydroxamic acid which was recrystallized from a suitable solvent.

### Preparation of labeled compounds

#### General procedure

The deuterated compounds were prepared by boiling



under reflux a solution of the appropriate hydroxamic acid (30-40 mg) in dioxane containing deuterium oxide (1-2 ml) for three to four hours. The mixture was then cooled and the product collected by filtration. Infrared and nuclear magnetic resonance (NMR) studies were used to assist in identification.

### 3-Amino-d<sub>2</sub>-1-hydroxy-d-2(1H)-quinolone

The title compound was prepared by the general procedure from 3-amino-1-hydroxy-2(1H)-quinolone. The IR spectrum of the latter compound showed N-H stretching bands at 3460 and 3360 cm<sup>-1</sup> which were replaced by two N-D stretching bands at 2590 and 2450 cm<sup>-1</sup> in the title compound. The O-H stretching band within the 3100-2000 cm<sup>-1</sup> region was replaced by an O-D stretching band at 2100-1800 cm<sup>-1</sup>. The NMR spectrum of the non-deuterated compound showed a 2-proton singlet at  $\tau$  4.42 for -NH<sub>2</sub> which was absent from the spectrum of the deuterated compound. The aromatic signal in the latter spectrum integrated for one proton less than for the non-deuterated compound indicating that the N-OH proton came to resonance in the aromatic region. These observations indicate that the hydrogen atoms of the amino and hydroxy groups were exchanged.

$M^+$  (mass spectrum): 179.

### 1-Hydroxy-d-2(1H)-quinolone-3-carboxylic acid-d

The general procedure was used to convert 1-hydroxy-







2(1H)-quinolone-3-carboxylic acid into the title compound. The IR spectrum of the non-deuterated compound showed a broad hydroxamate O-H stretching band in the 3400-2200  $\text{cm}^{-1}$  region which was replaced by an O-D stretching band in the 2480-1860  $\text{cm}^{-1}$  region in the spectrum of the title compound. The aromatic signal in the NMR spectrum of the non-deuterated acid integrated for 6 protons indicating that the N-OH proton was lost in this signal and the 1-proton singlet located at  $\gamma$  1.09 (COOH) in the NMR spectrum of the non-deuterated acid was absent from the spectrum of the title deuterated compound.

M<sup>+</sup> (mass spectrum): 207.

#### 2-Hydroxy-d-1(2H)-isoquinolone

The title compound was prepared by recrystallizing (three times) 2-hydroxy-1(2H)-isoquinolone from deuterium oxide. The O-H stretching band in the 3300-2000  $\text{cm}^{-1}$  region of the IR spectrum of 2-hydroxy-1(2H)-isoquinolone was replaced by an O-D stretching band in the 2300-1800  $\text{cm}^{-1}$  region in the title compound. The N-OH proton of the non-labeled compound was included in the aromatic region and the signal integrated for 1-proton less in this region for the title compound.

M<sup>+</sup> (mass spectrum): 162.

#### Ethyl 3,4-dihydro-3-d-1-hydroxy-d-2(1H)-quinolone-3-carboxylate

The title compound was prepared by the general pro-



cedure from ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate. The O-H stretching band in the 3700-2200  $\text{cm}^{-1}$  region of the latter was replaced by an O-D stretching band in the 2500-1800  $\text{cm}^{-1}$  region of the title compound. In the non-deuterated compound, the ring methylene 2-proton signal was a doublet ( $\gamma$  6.79 and 6.92) which was replaced by a 2-proton singlet ( $\gamma$  6.87) in the title compound. The NMR spectrum of the title compound also integrated for one proton less in the aromatic region indicating that the N-OH proton was included in this region in the non-deuterated compound, ethyl 3,4-dihydro-1-hydroxy-2(1H)-quinolone-3-carboxylate.

$\underline{M}^+$  (mass spectrum): 237.

#### 4-Hydroxy-d-2-methyl-d<sub>3</sub>-quinazoline-3-oxide

Treatment of 4-hydroxy-2-methylquinazoline-3-oxide according to the general procedure gave the title compound. The O-D stretching in the 2100-1800  $\text{cm}^{-1}$  region of the IR spectrum of the title compound replaced the O-H stretching band in the 2900-2000  $\text{cm}^{-1}$  region of the non-deuterated compound. The NMR spectrum of the non-deuterated compound contained a 3-proton singlet at  $\gamma$  7.28 ( $\underline{\text{CH}_3}$ ) and a 1-proton singlet at  $\gamma$  2.7 ( $\underline{\text{OH}}$ ) which were absent from the spectrum of the title compound.

$\underline{M}^+$  (mass spectrum): 180.



2-n-Butyl-3,4-dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzoxazine

The general procedure was used to prepare the title compound from 2-n-butyl-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine. The IR spectrum of the title compound showed an O-D stretching band at  $2290\text{ cm}^{-1}$  whereas the non-deuterated compound had an O-H stretching band at  $3100\text{ cm}^{-1}$ . An NMR spectrum of this compound was not done.

$\underline{M}^+$  (mass spectrum): 222.

3,4-Dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzoxazine

The general procedure was used to give the title compound from 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzoxazine. The IR spectra showed that the O-H stretching of the non-deuterated compound in the  $3400\text{--}2300\text{ cm}^{-1}$  region was replaced by an O-D stretching band at  $2290\text{ cm}^{-1}$  in the title compound. The NMR 1-proton signal for N-OH at  $\tau -0.63$  was absent in the spectrum of the title compound.

$\underline{M}^+$  (mass spectrum): 166.

7-Chloro-3,4-dihydro-4-d-3-oxo-2H-1,4-benzoxazine

The title compound was prepared from 7-chloro-3,4-dihydro-3-oxo-2H-1,4-benzoxazine by the general procedure. The 2-proton singlet at  $\tau 5.41$  in the NMR spectrum of the non-deuterated compound ( $\text{CH}_2$ ) was also present in the title compound. The NH proton was the exchangeable proton.



$\underline{M}^+$  (mass spectrum): 185 (13.2%), 187 (3.1%).

3,4-Dihydro-4-hydroxy-d-2-methyl-3-oxo-2H-1,4-benzothiazine

The title compound was prepared from 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine by the general procedure. The O-H stretching band at  $3200\text{ cm}^{-1}$  in the IR spectrum of the non-deuterated compound was replaced by an O-D stretching band in the IR spectrum of the title compound at  $2480\text{ cm}^{-1}$ . The NMR spectrum showed that the N-OH proton was included in the aromatic signal. The labeled compound integrated for one proton less than the non-deuterated compound in this region.

$\underline{M}^+$  (mass spectrum): 196.

3,4-Dihydro-4-hydroxy-d-3-oxo-2H-1,4-benzothiazine-2-d<sub>2</sub> 1,1 dioxide

The title compound was prepared from 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide according to the general procedure. The O-H stretching band in the IR spectrum of the non-deuterated compound in the  $3600\text{--}2300\text{ cm}^{-1}$  region was replaced by O-D stretching in the  $2500\text{--}2000\text{ cm}^{-1}$  region in the spectrum of the deuterated compound. The NMR spectrum of the non-deuterated compound showed a 2-proton singlet at  $\tau$  5.08 ( $\underline{\text{CH}_2}$ ) and a 1-proton singlet at  $\tau$  2.60 (N-OH), both of which were absent from the spectrum of the title compound.

$\underline{M}^+$  (mass spectrum): 216.







### PART III

#### ANTIBACTERIAL PROPERTIES



## ANTIBACTERIAL PROPERTIES

A property of some cyclic hydroxamic acids is their ability to inhibit the growth of bacteria. Since the discovery of the antibacterial properties of aspergillilic acid in 1940 (White and Hill, 1943), many hydroxamic acids have been synthesized and tested for their antimicrobial activity. It has been known for some time that 1-hydroxy-2(1H)-quinolone (21) possessed antibacterial properties (Newbold and Spring, 1948; Lott and Shaw, 1949). Coutts et al in 1965 reported that the nature of the substituents at positions 3- and 4- on the quinoline nucleus influences the antibacterial properties. From the limited number of examples examined, it seemed that an alkyl group is preferred at position 3 for optimal antibacterial activity. The most active compound tested was 1-hydroxy-3-methyl-2(1H)-quinolone (73). Because of these results it seemed desirable to prepare other quinoline derivatives and to compare their antibacterial properties with 1-hydroxy-2(1H)-quinolone and the 3-methyl derivative.

The initial screening was done qualitatively simply by streaking agar plates with S. aureus<sup>1</sup> and E. coli<sup>2</sup> and adding a few crystals of the compounds to the surface. After incubating at 37° for 24 hours, the zones of inhibition were noted and recorded (Table III).

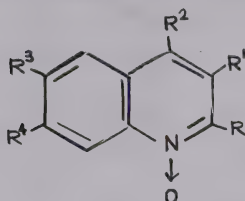
From the tabulated results it is significant to note that the hydroxamic acid grouping is necessary for activ-

1. Strain F.D.A. 209 (A.T.C.C. 6538) - a Gram positive organism.
2. Strain No. 402 (Dept. of Microbiology, University of Alberta) - a Gram negative organism.

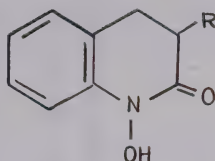


Table III

Initial Screening for Antibacterial Activity  
Against *S. aureus* and *E. coli*<sup>1</sup>



I



II

Compound	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	<i>S. aureus</i>	<i>E. coli</i>
Ia <sup>2</sup>	OH	CH <sub>3</sub>	H	H	H	-	-
Ib	OH	CH <sub>3</sub>	H	H	CH <sub>3</sub>	-	-
Ic	OH	CH <sub>3</sub>	H	CH <sub>3</sub>	H	-	-
Id	OH	NH <sub>2</sub>	H	H	H	-	+
Ie	OH	NO <sub>2</sub>	H	H	H	-	-
If <sup>2</sup>	OH	COOH	H	H	H	-	-
Ig <sup>2</sup>	OH	COOEt	H	H	H	-	-
Ih	OH	H	NO <sub>2</sub>	H	H	-	-
Ii <sup>2</sup>	OH	H	OH	H	H	+	-
Ij	OH	Br	OH	H	H	-	-
Ik	OH	H	Cl	H	H	-	-
Il	OH	H	CH <sub>3</sub>	H	H	-	-
Im	OH	H	H	CH <sub>3</sub>	CH <sub>3</sub>	-	-
In <sup>2</sup>	H	CH <sub>3</sub>	H	H	H	+	+

<sup>1</sup> A negative (-) sign in Tables III-VI indicates inhibition of growth; a positive (+) sign indicates that there was no zone of inhibition.

<sup>2</sup> Antibacterial activity reported by Coutts et al, 1965.



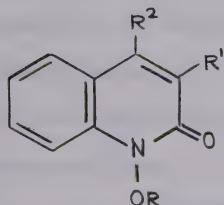
Table III cont'd...

Compound	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	<u>S. aureus</u>	<u>E. coli</u>
Io	H	CH <sub>3</sub>	H	H	CH <sub>3</sub>	+	+
Ip	H	CH <sub>3</sub>	H	CH <sub>3</sub>	H	+	+
Iq	H	NO <sub>2</sub>	H	H	H	-	-
Ir <sup>3</sup>	H	H	NO <sub>2</sub>	H	H	-	-
Is	H	H	Cl	H	H	+	-
It	Cl	H	NO <sub>2</sub>	H	H	-	-
Iu	H	H	H	CH <sub>3</sub>	CH <sub>3</sub>	+	+
Ii	CN	-	-	-	-	-	-

<sup>3</sup> Antibacterial activity reported by Arai and Nakayama, 1952; Okabayashi, 1953.

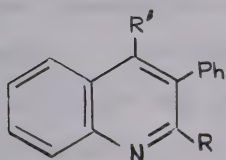




Table IVAntibacterial Screening of 1-substituted-2-(1H)-quinolones

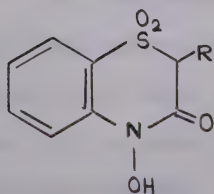
III

R	R <sup>1</sup>	R <sup>2</sup>	<u>S. aureus</u>	<u>E. coli</u>
COCH <sub>3</sub>	CN	H	+	+
CH <sub>2</sub> Ph	H	H	+	+

Table VAntibacterial Screening of Quinoline Derivatives

IV

R	R <sup>1</sup>	<u>S. aureus</u>	<u>E. coli</u>
OH	OH	+	+
Cl	Cl	+	+

Table VIAntibacterial Screening of Some 3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxides

V

R	<u>S. aureus</u>	<u>E. coli</u>
H	+	+
CH <sub>3</sub>	+	+
Br	-	-



ity in all compounds except the 4-nitro-derivatives. 4-Nitroquinoline 1-oxide has been shown to have a broad spectrum of activity by various workers (Arai and Nakayama, 1952; Okabayashi, 1953; Leonard et al, 1956). Thus, it is not surprising that 2-chloro-4-nitroquinoline 1-oxide exhibits activity.

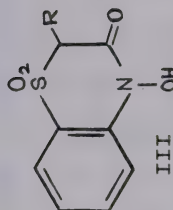
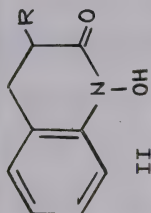
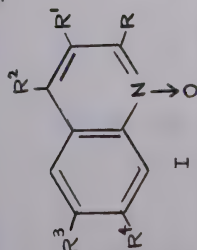
It is interesting to note that the addition of a halogen to a known hydroxamic acid results in increase of activity. 3-Bromo-1,4-dihydroxy-2(1H)-quinolone has broader activity than the 1,4-dihydroxy-derivative. The same observation was noted in the benzothiazine 1,1-dioxide series. 3,4-Dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide and 3,4-dihydro-4-hydroxy-2-methyl-3-oxo-2H-1,4-benzothiazine 1,1-dioxide did not create a zone of inhibition. Replacing the methyl grouping with a bromine atom to give 2-bromo-3,4-dihydro-4-hydroxy-3-oxo-2H-1,4-benzothiazine 1,1-dioxide resulted in a compound which gave zones of inhibition against both S. aureus and E. coli.

The antibacterial activity of any compound varies with the testing method used. Antibiotics, notably penicillin, have been compared quantitatively with a standard for purposes of estimation of their potency, by the diffusion method. Because an aqueous solution of hydroxamic acids can be easily prepared, it seemed desirable to test initially the activity of the antibacterial agents by measuring the zones of inhibition that result from the



Table VII

Antibacterial Activity Obtained by  
the Diffusion Method<sup>1</sup>



Compound	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	S. aureus		E. coli	
						mg %	zone (mm)*	mg %	zone (mm)
Ia	OH	H	H	H	H	20	9.4	50	10.3
Ib	OH	CH <sub>3</sub>	H	H	H	-	-	50	12.0
Ic	OH	CH <sub>3</sub>	H	CH <sub>3</sub>	H	36	10.7	-	-
Id	OH	CH <sub>3</sub>	H	H	CH <sub>3</sub>	26	9.9	-	-
Ie	OH	NO <sub>2</sub>	H	H	H	-	-	-	-
If	OH	NH <sub>2</sub>	H	H	H	32	10.1	-	-
Ig	OH	Br	OH	H	H	50	9.1	-	-
Ih	OH	H	H	CH <sub>3</sub>	CH <sub>3</sub>	40	12.2	32	10.6
Ii	OH	H	H	NO <sub>2</sub>	H	40	13.3	40	10.4



Table VII cont'd...

Compound	R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	S. aureus		E. coli	
						mg %	zone (mm)	mg %	zone (mm)
Ij	OH	H	Cl	H	H	-	-	-	-
Ik	OH	H	NO <sub>2</sub>	H	H	-	-	20	13.2
Il	OH	H	CH <sub>3</sub>	H	H	-	-	-	-
Im	H	NO <sub>2</sub>	H	H	H	50	9.8	10	12.0
In	H	H	Cl	H	H	-	-	-	-
Io	Cl	H	NO <sub>2</sub>	H	H	-	-	-	-
IIa	CN	-	-	-	-	36	10.2	-	-
IIb	Br	-	-	-	-	-	-	-	-
Aspergilllic acid						48	12.4	-	-
Nitrofurazone						-	-	20	12.2

\* Zones were measured with a Fisher-Lilly Antibiotic Zone Reader.

1. Porcelain Penicylinders<sup>®</sup> (Fischer Scientific Co., Catalogue No. 7-907) were used.





diffusion of the aqueous solution from a cylindrical cup placed on the agar test plate.

Aqueous solutions were prepared and dilutions were made so that a range of 10-50 mg % could be examined. Table VII gives the measurement of the zone of inhibition for the minimum inhibitory concentration.

The diffusion method for measuring antibacterial activity is a fairly rigorous method but it is a good method for screening a number of potentially active antibacterial compounds. A compound which shows activity by this method is a fairly effective antibacterial agent. The antimicrobial results of the hydroxamic acids and N-oxides tested (Table VII) compare favorably with those obtained with aspergilllic acid and nitrofurazone.

Some of the hydroxamic acids studied by this method seem to be quite active, notably 3,6-dimethyl- and 3,7-dimethyl- 2(1H)-quinolones. It was decided that these and the other active compounds should be subjected to further testing. The tube dilution method was chosen because it was thought that a better picture of the in vitro antibacterial activity could be obtained in this way. The antibacterial agent is in direct contact with the bacteria and does not depend on diffusion before it comes in contact.

Initially, a method similar to that outlined by Vickers (see Coutts et al, 1965) was tried. Inhibition of growth was obtained but a great deal of difficulty was



encountered. The results were hard to reproduce. This could be due to the large amount of bacteria (0.2 ml of a 24 hour culture in 30 cc broth) added to each tube. The minimum inhibitory concentration<sup>(m.i.c.)</sup> of an antibacterial agent may increase greatly with an increase in the concentration of bacteria used in the test because there is an increase in the number of resistant bacteria. When large inocula are used, the bacteria that actually function in the test are the few that are resistant and not the greater part of the population that are sensitive to the active substance (Kavanagh, 1963).

A procedure outlined by Kavanagh (1963)<sup>1</sup> was the method next employed. It was found to be a very satisfactory method giving reproducible results. Tables VIII and IX give the minimum inhibitory concentrations obtained in milligram percent for each compound tested.

Table VIII

<sup>2</sup>  
Antibacterial Activity of Various Quinoline  
1-oxides (m.i.c. in mg %)

Substituent	<u>S. aureus</u>	<u>E. coli</u>
3-nitro-	7.84	3.96
4-nitro-	0.25	0.13
5-nitro-	>16	9.75
6-nitro-	>16	>16
2-chloro-4-nitro-	0.995	1.96

<sup>1</sup> See method C, p. 215 of this thesis.

<sup>2</sup> See footnote to Table IX.



Table IX

Antibacterial Activity<sup>1</sup> of Various 1-hydroxy-  
2(1H)-quinolones (m.i.c. in mq %)

Substituent	<u>S. aureus</u>	<u>E. coli</u>
3-methyl-	7.84	3.92
3,6-dimethyl-	3.92	3.92
3,7-dimethyl-	2.95	1.98
3-amino-	> 16	11.82
3-cyano-	> 16	> 16
3-nitro-	13.52	5.91
6,7-dimethyl-	15.69	11.82
4-methyl-	7.84	2.95
4-nitro-	7.88	1.99
5-nitro-	> 16	> 16
6-nitro-	> 16	> 16
4-chloro-	15.69	15.69
Aspergillic acid	7.92	3.98
1-hydroxy-2(1H)- quinolone	> 40	27.05
3-cyano-3,4-dihydro- 1-hydroxy-2(1H)- quinolone	5.91	15.69

<sup>1</sup> Solutions containing not greater than 5% v/v ethanol were examined. Controls were also examined.



From the few nitro-quinoline 1-oxides studied (Table VIII) it is seen that the presence of a substituent in the 4-position is the most desirable feature for activity. The activity greatly decreases when there is substitution in the benzene portion of the nucleus. Leonard et al (1956) observed in testing 4-substituted N-oxides that the 4-nitro substituent gave a very active compound.

From Table IX it is noted that alkyl groups in the 3- and 4- positions gives a hydroxamic acid which is active against Gram positive and Gram negative bacteria. 3-Methyl-, 3,6-dimethyl-, 3,7-dimethyl- and 4-methyl- 2(1H)quinolones are all active in fairly low concentrations. The results compare favorably with the activity of aspergillic acid and are superior to those of the parent unsubstituted hydroxamic acid, 1-hydroxy-2(1H)-quinolone.

It is observed that the nitro-hydroxamic acids, like the N-oxides, exhibit the greatest activity when the nitro group was in position 4- of the nucleus. Moving the nitro substituent to the benzene portion of the nucleus seemed to greatly decrease the activity.

It is of interest to compare the activities of 3-cyano-1-hydroxy-2(1H)-quinolone and 3-cyano-3,4-dihydro-1-hydroxy-2(1H)-quinolone. The latter displays much greater activity against S. aureus and is more active against E. coli. The fact that the latter hydroxamic acid is more active could mean that planarity of the molecule is not the most desirable feature for activity. Perhaps partially re-





duced cyclic hydroxamic acids would possess even better antibacterial activity if they were methyl-substituted compounds. This feature will have to be studied further.

### Activity of the Sodium Salts

Three aqueous solutions, prepared for the studies by the diffusion method, were<sup>next</sup> tested by the tube dilution method described by Kavanagh (1963). 3-Cyano-3,4-dihydro-1-hydroxy-2(1H)-quinolone, 1-hydroxy-3,7-dimethyl-2(1H)-quinolone and aspergillilic acid did not inhibit the growth of the bacteria (neither S. aureus nor E. coli) at a concentration of 15 mg %. These results are disappointing because an aqueous solution would be better for testing than an alcoholic solution as alcohol is antibacterial itself at certain concentrations. The fact that these compounds are less active may be due to ionization. Albert (1968) when reporting on the studies done on oxine points out that any ionization of oxine results in decreased activity. For example, 8-hydroxyquinoline-5-sulfonic acid does not alter the chelating property in vitro, but the antibacterial activity is lost, presumably due to the inability of the ion to penetrate the bacterial cell wall.

### Bacteriostatic and Bacteriocidal Properties

The compounds prepared for this antibacterial study were found to be effective at low concentrations. It was of interest to see if the antibacterial activity was due to bacteriostasis or if the bacteria were actually being



killed.

A loopful of solution was taken from the tubes which had complete inhibition of growth and added to a tube containing broth so that the antibacterial agent would be greatly diluted. After twenty four hours of incubation at 37°, the tubes were observed for cloudiness. If the tubes were clear a loopful of this dilution was again added to more broth and incubated for twenty four hours at 37°. If there was still no growth, bacteria were added to the tube to be sure that the antibacterial agent was not in such a concentration as to inhibit growth. If, however, the subcultured tubes were cloudy, a loopful was plated on an agar plate to be sure the cloudiness was due to bacterial growth.

When a high concentration of bacteria (Vickers method ref. 43) was used it appeared that the compounds were bacteriostatic. Cloudiness was obtained on subculturing in all cases and plating it out on an agar plate proved that the cloudiness was due to bacterial growth.

When the procedure described by Kavanagh was employed to evaluate the antibacterial agents, no cloudiness was produced after subculturing except in the case of 4-chloro-1-hydroxy-2(1H)-quinolone (against S. aureus at 15.69 mg %) which was shown to be bacteriostatic at this concentration. The other compounds did not have any growth on subculturing the second time. Thus, when the bacterial concentration was low the compounds examined in this study were bacteriocidal.



## Experimental

Dr. R.T. Coutts provided the sample of 3-bromo-1,4-dihydroxy-2(1H)-quinolone. All the other compounds have been mentioned previously in this thesis.

### Cup-plate Method (Diffusion method)

Aqueous solution of the hydroxamic acids were prepared by suspending the hydroxamic acid (10 mg) in a small amount of water and adding sufficient 0.01N sodium hydroxide to facilitate solution. The solutions were back-titrated with 0.01N hydrochloric acid to pH 7 (potentiometrically) unless the hydroxamic acid began to precipitate out. If the latter was the case the back titration was taken only to the point where cloudiness began to appear. The solutions were then diluted to 10 ml with water. Serial dilutions were made from these solutions. The minimum and maximum concentrations of the substances tested were 10 and 50 mg %.

The N-oxides were dissolved in hydrochloric acid and back titrated with 0.01N sodium hydroxide solution to pH 7 and then treated as above.

The solutions (0.2 ml) were added to cylindrical cups placed on top of the agar plate. The plates were made by pouring base agar (20 ml) into a petri dish and after it solidified seed agar (8 ml), inoculated with a twenty four hour culture of bacteria (0.2 ml), was added. The plates were incubated at 37°C for twenty four hours and observed



for zones of inhibition.

### Tube Dilution Method

Method A (Vickers method described in Coutts et al, 1965)

Serial dilutions of a solution of the compound (4 mg) in alcohol (0.5 ml) were added to tubes of nutrient broth (9.3 ml). The test organism was then added (0.2 ml of a 4 day culture) and the tubes incubated for twenty four hours and inspected for inhibition of growth.

### Method B

Method A was repeated except the test organism employed was a twenty four hour culture.

Method C (Kavanagh, 1963)

The inoculum was prepared by transferring a large number of bacteria (a loopful) from an agar plate to a tube of antibiotic assay broth (9.3 ml) and incubating it for six hours at 37°C. The tubes were shaken occasionally to aerate and to promote growth. The six hour broth cultures of the bacteria were then diluted one million times in the antibiotic broth to give a concentration of bacteria from 500-2000/ml (Kavanagh, 1963).

The antibacterial testing was done by adding serial dilutions of a solution of the compound (4 mg) in alcohol (0.5 ml) to broth (0.5 ml) and then adding the inoculated broth (0.5 ml) to each tube. The tubes were incubated at 37°C and observed for growth at sixteen to eighteen, twenty four and forty two hour intervals. Appropriate controls were set up.







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